REMARKS

Claims 1-86 are pending in the application. Claims 1-24, 29, 31-44, 51-54, 59, 60, 66-80, and 82-86 are withdrawn. Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are rejected under 35 U.S.C. § 112, first paragraph, as failing to meet the written description requirement. Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are further rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are also rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Siegel et al. (U.S. Patent No. 6,204,245; hereafter "Siegel") or over Shi et al. (U.S. Application Publication No. 2002/0012966; hereafter "Shi"). Lastly, claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 22-41, 49-53, and 70 of copending Application Serial No. 10/670,488. Applicants address each of these rejections below.

Claim Amendments

Claims 22, 23, 27, 29, and 51-65 have been canceled. Claims 25, 26, 31, 44, and 81 have been amended. Support for the present amendment is found in the previously pending claims and in the specification at pg. 9, lines 4-13; pg. 38, lines 11-18; pg. 38, line 27, to pg. 39, line 4; pg. 39, lines 12-26; and pg. 40, lines 5-9. No new matter has been added by the present amendment. Applicants reserve the right to pursue any canceled subject matter in a continuing application.

Rejections under 35 U.S.C. § 112, first paragraph

Written Description

Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are rejected under 35 U.S.C. § 112, first paragraph, for failure to meet the written description requirement. As the basis for

this rejection, the Office asserts that the terms "analog," "glucocorticoid receptor modulator," "small molecular immunomodulator," and "biologic" do not suffice to meet the written description requirement.

Applicants have amended claims 25 and 26 to remove the term "analog;" amended claims 31 and 81 to specify that the biologic is selected from the group of "alefacept, inflixamab, adelimumab, efalizumab, etanercept, CDP-870, rituximab, atlizumab, and omalizumab" and to specify that the small molecular immunomodulator is selected from the group of "p38 MAP kinase inhibitors, TACE inhibitors, ICE inhibitors, and IMPDH inhibitors;" and have canceled claims 51-65 reciting the term "glucocorticoid receptor modulator." Applicants submit that that the rejection under 35 U.S.C. § 112, first paragraph, for written description may now be withdrawn.

Enablement

Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. As the basis for this rejection, the Office states that "while being enabling for reduction of certain secretions *in vitro*, [the specification] does not reasonably provide enablement for treating the broader immunoinflammatory disorders..., or decreasing cytokine secretion..." (Office Action, pg. 5).

In response, Applicants have amended claim 26 to recite the following immunoinflammatory disorders: rheumatoid arthritis, psoriasis, ulcerative colitis, Crohn's disease, ankylosing spondylitis, multiple sclerosis, polymylagia rheumatica, and giant cell arteritis. Applicants submit that the specification contains data that demonstrates the synergistic effect of the combination of a corticosteroid and an SSRI on decreasing the secretion of the proinflammatory cytokine, TNFα, and that given these data and the role of TNFα in the pathogenesis of the presently claimed immunoinflammatory disorders and the effective use of TNFα for the treatment of the majority of these disorders, this rejection should be withdrawn.

The specification contains data showing the synergistic effect of the combination of an SSRI and a corticosteroid on decreasing TNFα secretion/production: paroxetine and prednisolone (Table 15, page 84; 0.375 μM paroxetine + 0.025 μM prednisolone); fluoxetine and prednisolone (Table 16, page 84; 7.23 μM fluoxetine + 0.006 μM prednisolone); fluoxetine and budesonide (Table 17, page 84; 0.009 μM fluoxetine + 0.009 μM budesonide); paroxetine and dexamethasone (Table 18, page 85; 3.0 μM paroxetine + 0.0063 μM dexamethasone); fluoxetine and dexamethasone (Table 19, page 85; 0.036 μM fluoxetine + 0.0024 μM dexamethasone); fluoxetine and prednisolone (Table 20, page 85; 1.80 μM fluoxetine + 0.0160 μM prednisolone); paroxetine and prednisolone (Table 21, page 86; 3.30 μM paroxetine + 0.016 μM prednisolone); and sertraline and prednisolone (Table 22, page 86; 4.0 μM sertraline + 0.0160 μM prednisolone). These data are also summarized in the enclosed Exhibit 1, where it is clearly shown that the combined effect of an SSRI with a corticosteroid results in an effect that is greater than the sum of the effects of the SSRI and corticosteroid when administered alone. Applicants submit that given the *in vitro* data, one skilled in the art would reasonably expect that administration of the combination of a corticosteroid and an SSRI would result in a decrease in the secretion or production of a proinflammatory cytokine (e.g., TNFα) *in vivo*.

Although not agreeing with the Office's position that the claimed methods are not enabled for the treatment of the broad class of immunoinflammatory disorders, Applicants have amended the claims to recite a specific subset of disorders to expedite prosecution of the application. Applicants reserve the right to pursue all canceled subject matter in a separate application. For the remaining disorders, namely, rheumatoid arthritis, psoriasis, ulcerative colitis, Crohn's disease, ankylosing spondylitis, multiple sclerosis, polymylagia rheumatica, and giant cell arteritis, Applicants summarize below the published data that establish the relationship between the inhibition of the proinflammatory cytokine, TNFα, and the treatment of each of the claimed disorders.

Applicants submit that given the data in the specification and the data that establish a relationship between the inhibition of TNFα and the treatment of each of the claimed immunoinflammatory disorders (discussed below), one skilled in the art would reasonably predict that the combination of a corticosteroid and an SSRI could be used to treat the presently claimed immunoinflammatory disorders (see, Declaration of Grant Zimmermann, Ph.D.).

As mentioned above, Applicants present below various lines of evidence that establish a relationship between the inhibition of TNFα and treatment of an immunoinflammatory disorder. Examples of the types of evidence that point to a role for TNFα in the pathogenesis of an immunoinflammatory disorder include the following:

> Type 1. An elevated level of TNFα that is found to correlate with the disorder or is found at the sites of inflammation (e.g., in the joints of mice with rheumatoid arthritis).
>
> Type 2. Demonstration of the disorder or an etiologically similar disorder in transgenic animals overexpressing TNFα.
>
> Type 3. Amelioration or prevention of the disorder with TNFα inhibitors.

The first two types of evidence can be used to provide the basis or the rationale for therapies aimed at reducing TNFα, while the third type of evidence, demonstration of the therapeutic efficacy of a known TNFα inhibitor for a particular disorder, provides the proof of principle that inhibition of TNFα can be used to treat the disorder.

Importantly, for some of the indications described below, the use of TNFα inhibitors has not only been shown to be effective, but *has been approved by the FDA* for the treatment of the disorder. Given the rigorous evaluation of the safety and efficacy data by a team of physicians, statisticians, chemists, pharmacists, and other scientists at the FDA, approval of a TNFα inhibitor for the treatment of a particular

disorder provides an almost indisputable correlation between inhibition of TNFα and treatment of the disorder.

Applicants note that, although there are several TNFα inhibitors that are under development for the treatment of immunoinflammatory disorders, only three FDA approved TNFα inhibitors are included in this summary. These are infliximab (REMICADE), which is a chimeric monoclonal antibody that binds TNFα and TNFβ; etanercept (ENBREL), which is a human TNF receptor protein dimer fused to human IgG1 that binds TNFα and TNFβ; and adalimumab (HUMIRA), which is a fully humanized anti-TNFα antibody.

Rheumatoid Arthritis

In support of enablement of the claimed methods for treatment of rheumatoid arthritis in a subject, Applicants provide evidence from all three categories listed above demonstrating that TNFα is elevated in rheumatoid arthritis (RA) and that inhibition of TNFα, through the use of all three recited TNFα inhibitors, is an effective and *FDA-approved* treatment for RA.

Several lines of evidence exist in animals models that support the importance of TNFα in the pathogenesis of human RA. Some of the findings include the detection of elevated levels of TNFα in the joints of mice with collagen-induced arthritis (CIA) and the prevention or amelioration of CIA with anti-TNF blocking antibodies. CIA is a widely accepted animal model for human RA and can be induced in several species by immunization with heterologous type II collagen (Durie et al., *Clin. Immunol. Immunopathol.* 73:11-18, 1994; Badger et al., *Arth. Rheum.* 43:175-183, 2000; and Shou et al., *Arth. Res. Ther.* 8:R28, 2006). In addition, inflammatory arthritis has been shown to develop in transgenic mice overexpressing TNFα.

TNFα inhibitors were first used in the U.S. for the treatment of RA in 1998. Since then, etanercept, infliximab, and adalimumab have been studied in human subjects, and

are now approved by the FDA for the treatment of RA. Several studies of these drugs are summarized below.

The initial studies included only patients with long-standing severe RA that had failed multiple conventional treatments. Many patients suffering from RA had rapid, positive clinical responses to all three TNFα inhibitors. More recently, patients with early disease have had positive responses to the TNFα inhibitors. In addition, radiographic data in both early and late disease patients demonstrated the ability of TNFα inhibitors to slow or halt the development of new or enlarging erosions and the development of new or progressive joint space narrowing in the majority of patients. These results have led to the widespread use of TNFα inhibitors as a standard treatment for RA.

The first approval of etanercept was based on studies demonstrating the therapeutic efficacy of the drug in patients having an inadequate response to one or more disease-modifying antirheumatic drugs (DMARDs). These studies all showed strong positive results for the patients treated with etanercept as compared to those receiving placebo and provided sufficient evidence to obtain FDA approval of etanercept for the treatment of RA.

The Early Erosive Rheumatoid Arthritis (ERA) study demonstrated that etanercept could be used to treat RA and modify disease progression in patients without any previous treatment with methotrexate, a conventional DMARD. The ERA trial was a Phase III randomized, double-blind, multi-center trial that included 632 adults who had early stage RA (less than three years and had never been treated previously with methotrexate). The positive data from this study were used to apply for and obtain FDA approval of etanercept for reducing the symptoms in patients with moderately to severely active RA in the early treatment of RA. The success of etanercept in this trial was significant because effective treatment early in the disease can prevent or reduce the chances of developing significant deformities and disabilities as the disease progresses.

The ongoing clinical benefits of etanercept for the treatment of RA have also been studied in patients using etanercept as monotherapy. For this study, 629 patients were observed in an open-label follow up study for five years. Evaluation of the ongoing clinical efficacy of etanercept demonstrated that the therapeutic benefits of etanercept are continued over time.

Infliximab was the second FDA-approved TNF inhibitor for the treatment of RA. There are numerous published studies demonstrating the efficacy of infliximab for the treatment of RA. The ATTRACT trial contributed to FDA approval of infliximab for the treatment of RA. The ATTRACT trial was a double blind, placebo-controlled, randomized trial of 428 patients at 34 clinical sites in North America and Europe. The data from the ATTRACT trial demonstrated a significant improvement in all infliximab-treated groups as compared to the placebo group. Analysis after one year of blinded therapy confirmed the statistical superiority of all methotrexate plus infliximab treatment groups compared to placebo.

The FDA approval of the third TNF inhibitor, adalimumab, for the treatment of RA was based, in part, on the ReAct trial in which 61% of patients who previously failed other anti-TNF therapies showed a clinically positive response with adalimumab. Adalimumab has subsequently been approved for first-line treatment of recent onset moderate to severe RA.

The literature is replete with studies demonstrating the efficacy of TNFα inhibitors in treating RA. A sample of these studies is summarized in Nanda et al. (*Expert. Opin. Pharmacother.* 5:1175-1186, 2004). Table 1 in Nanda et al. summarizes five studies that demonstrate the efficacy of etanercept as compared to placebo for reducing symptoms of RA. Another example can be found in Edwards et al. (*Ann. Rheum. Dis.* 58 (Suppl. I):I73-I81, 1999) which describes a study showing that infliximab has significant effects in short-term and long term duration in clinical trials in RA patients. Moreland et al. (*Ann. Intern. Med.* 130:478-486, 1999) describes a study showing that etanercept

significantly reduced disease activity in a dose-related fashion in patients with RA in a randomized double blind, placebo controlled trial.

The proven clinical efficacy of three different TNFα inhibitors for the treatment of RA combined with the FDA approval of all three TNFα inhibitors for the treatment of RA demonstrates the role of TNFα in the pathogenesis of the disease and the importance of TNFα inhibitors for the treatment of RA.

In sum, Applicants have provided ample data supporting a clear basis for the translation of TNFα inhibition to the treatment of RA that includes evidence from all three types of evidence cited above. This evidence includes the detection of elevated levels of TNFα in the joints of mice with collagen-induced arthritis (CIA) (Type 1) and the finding that inflammatory arthritis has been shown to develop in transgenic mice overexpressing TNFα (Type 2). The FDA approval of three TNFα inhibitors for the treatment of RA (Type 3) underscores the scientifically accepted finding that inhibition of TNFα is an effective and widely used therapeutic modality for the treatment of RA.

In view of the above, Applicants submit the above evidence demonstrating a role for TNFα in the pathogenesis of RA and the efficacy of anti-TNFα agents for the treatment of RA is sufficient to establish that the proven synergistic effects of the combination of a corticosteroid and a SSRI against TNFα secretion would reasonably be expected to translate into an effective treatment for RA. Applicants respectfully request that the enablement rejection, as it applies to RA, be withdrawn.

Psoriasis

In support of enablement of the claimed methods for treatment of psoriasis in a subject, Applicants provide evidence that TNFα is elevated in human patients with psoriasis and that inhibition of TNFα, through use of all three recited TNFα inhibitors, is an effective and *FDA-approved* treatment for psoriasis.

Evidence for the role of TNFα in the pathogenesis of psoriasis includes the detection of higher levels of TNFα immunoreactivity and biological activity in lesions from patients with psoriasis. For example, Krueger et al. (*Arch. Dermatol.* 140:218-225, 2004) describe the finding that the production of TNFα by peripheral blood mononuclear cells from patients with active psoriasis was positively linked with clinical severity, and that levels of TNFα in psoriatic skin blister fluids are significantly correlated with Psoriasis Area and Severity Index (PASI) scores. These studies link TNFα levels with the severity of symptoms of psoriasis.

Ettehadi et al. (*Clin. Exp. Immunol.* 96:146-151, 1994) analyzed aqueous extracts from psoriatic skin lesions for the presence of TNFα immunoreactivity and biological activity, and found that TNFα immunoreactivity and biological activity were consistently higher in lesions as compared with uninvolved samples. These results demonstrate that TNFα is elevated in patients with psoriasis, particularly at the active lesions.

The first TNFα inhibitor to be approved for the treatment of moderate-to-severe psoriasis was etanercept. Studies of the efficacy of etanercept in patients with psoriasis demonstrated that nearly half of patients (i.e., 47% in study I and 46% in study II) taking etanercept experienced 75% or better improvement in psoriasis severity.

On September 27, 2006, the FDA approved infliximab for the treatment of severe plaque psoriasis. In one Phase III clinical trial (the EXPRESS trial), 80% of patients receiving infliximab achieved 75% improvement in psoriasis versus 3% of patients receiving placebo. Similar results were seen with EXPRESS II, the second Phase III study.

In 2008, adalimumab also received FDA approval for the treatment of adult patients with moderate-to-severe chronic plaque psoriasis. The approval was based on two pivotal clinical trials, REVEAL and CHAMPION, showing that nearly 3 in 4 patients achieved 75% clearance or better at week 16 of treatment versus placebo.

Additional studies demonstrate the efficacy of TNFα inhibitors for the treatment of psoriasis. For example, in a Phase II study using etanercept for the treatment of patients with chronic plaque psoriasis, 56% showed a 75% improvement in Psoriasis Area and Severity Index (PASI) score and a 75% response in lesion clearing as compared with only 5% and 4%, respectively, of the placebo-treated patients.

The results of these studies, along with the approval by the FDA of three TNFα inhibitors for treatment of psoriasis, demonstrate that inhibition of TNFα is an effective therapeutic modality for the treatment of psoriasis.

Applicants submit the above evidence demonstrating a role for TNFα in the pathogenesis of psoriasis and the efficacy and *FDA-approval* of anti-TNFα agents for the treatment of psoriasis is sufficient to establish that the synergistic effects of Applicants' combination of a corticosteroid and a SSRI would reasonably be expected to translate into an effective treatment for psoriasis. Applicants respectfully request that the enablement rejection, as it applies to psoriasis, be withdrawn.

Ulcerative Colitis

In support of enablement of the claimed methods for treatment of ulcerative colitis (UC) in a subject, Applicants provide evidence that TNFα is elevated in UC and that inhibition of TNFα, through use of three TNFα inhibitors (including one that is FDA-approved for the treatment of UC) is an effective therapeutic against UC.

There is evidence in the literature demonstrating an elevation in TNFα levels in inflammatory bowel diseases (IBDs) in general (see for example, Ardizzone et al., *J. Intern. Med.* 252:475-496, 2002; and Lichtenstein et al., *Inflammatory Bowel Diseases* 7:89-93, 2001). Additional studies report increased levels of TNFα in UC specifically (see, Shen et al., *J. Clin. Gastroenterol.* 38:741-745, 2004; and Lichtenstein et al., *supra*). In one particular reference, Murch et al. (*Gut* 34:1705-1709, 1993) describe their findings that TNFα-immunoreactive cells are present at high density in the lamina propria in both

IBDs, Crohn's Disease (CD), and UC, and within the submucosa in CD, and conclude that these results support the use of TNFα inhibitors for the treatment of IBDs in general.

There is also an animal model, described in Shen et al. (*supra*) and Lichtenstein et al. (*supra*), that supports the role for TNFα in the pathogenesis of UC. This animal model is a cotton-top tamarin, which spontaneously develops UC with complications similar to those found in human UC. TNFα levels are increased in the feces of these animals and CPD571, a chimeric anti-TNFα antibody similar to infliximab, has been shown to produce rapid clinical and histological improvement in these animals. These results show the correlation between TNFα levels and UC in humans and in an animal model, providing evidence under types 1 and 2 above, and suggest that TNFα inhibitors may be effective for the treatment of UC.

Several trials evaluating the use of TNFα inhibitors for the treatment of UC provided the foundation for the development of two clinical trials that led to the approval of infliximab for the treatment of UC. In one of the trials, conducted by Sands et al. (*Acta. Gastro-Enterologica Belgica* 64:205-209, 2001), 50% of patients who received infliximab showed clinical response, while none of the three patients receiving placebo had a response. The trial showed promise, but was terminated due to slow recruitment of patients. Shen et al. (*supra)* and Lichtenstein et al. (*supra)* summarize several studies examining the current therapeutic approaches to UC, including the use of TNFα inhibitors. For example, Table 1 of Shen et al. lists 10 separate studies using infliximab for the treatment of UC and indicates that the response rate ranges from 39% to 100% in the various randomized, double-blind placebo, and retrospective studies. Shen et al. postulate that the cytokines involved in UC may vary in some patients and that clinical improvement seen with TNFα inhibitors may occur in those patients having an overproduction of TNFα. Lichtenstein et al. (*supra)* also summarizes studies of infliximab for the treatment of UC and concludes that, despite the small number of

patients in the trial, the results suggest that infliximab was well-tolerated and may provide clinical benefit to patients with severe, steroid-refractory UC.

In September of 2005, infliximab was approved by the FDA for the treatment of UC based on positive results from two randomized, placebo-controlled, pivotal Phase III clinical trials, ACT 1 and ACT 2, to evaluate the safety and efficacy of infliximab in patients with moderate to severely active UC. In each trial, 364 patients with active UC who were unresponsive to at least one standard therapy were enrolled. In ACT 1, significantly higher proportions of patients receiving infliximab achieved clinical response at week 8 and at week 30 versus patients receiving placebo infusions. Mucosal healing was also achieved in significantly more patients receiving infliximab than patients receiving placebo infusions.

In ACT 2, significantly higher proportions of patients receiving infliximab achieved clinical response at week 8 and week 30 versus patients receiving placebo infusions.

The evidence for a role for TNFα in IBDs, in general, and in UC specifically, can be found throughout the literature. Taken together with the strong positive results and the FDA approval of infliximab for the treatment of UC, these findings support the assertion that inhibition of TNFα is a clinically proven therapeutic modality for treating UC. Applicants submit that this evidence is sufficient to establish a role for TNFα in UC and that the proven synergistic effects of Applicants' claimed combination of a corticosteroid and an SSRI would reasonably be expected to translate into an effective treatment for UC. Applicants respectfully request that the enablement rejection, as it applies to UC, be withdrawn.

Crohn's Disease

In support of enablement of the claimed methods for treatment of Crohn's Disease (CD) in a subject, Applicants provide evidence that TNFα is elevated in CD and that

inhibition of TNFα is an effective therapy against CD. Applicants note that two of the TNFα inhibitors have already been *FDA-approved* for the treatment of CD. These results are discussed in detail below.

CD is characterized by mucosal inflammation with enhanced expression of TNFα and chemokines, and destruction of the epithelial lining (see Schreiber et al., *Lancet* 353:459-461, 1999). There is evidence in the literature that TNFα is elevated in CD and may be critical for the mucosal inflammation seen in IBDs and in CD specifically. Braun et al. (*Ann. Rheum. Dis.* 61(Suppl. III):iii51-iii60, 2002) summarize some of the findings regarding TNFα and CD in Table 1, and state that *in vitro* studies have shown that TNFα is increased in the mucosa of patients with CD. In addition, Ardizzone et al. (*supra*) state that IL-1 and TNFα appear to be critical to the amplification of mucosal inflammation in IBDs, and Figure 1 of Ardizzone et al. shows TNFα as a key cytokine involved in the development of CD. As described above for UC, Murch et al. (*supra)* studied the distribution and density of TNFα containing cells in the mucosa of patients with IBD and found that TNFα-immunoreactive cells are present at high density in the lamina propria in both CD and UC, and within the submucosa in CD. Murch et al. conclude from their study that therapeutic inhibition of TNFα may provide potent and selective options for the treatment of CD. All of these studies demonstrate a correlation between elevated TNFα levels and IBDs, particularly CD, in humans.

Nikolaus et al. (*Lancet* 356:1475-1479, 2000) examined the treatment of refractory CD with infliximab in patients in whom remission was short-lived and in whom there was a possible link with TNFα levels. Their studies were based on the finding that a single application of infliximab induced rapid clinical improvement in most patients, but that remission in these patients was sometimes short-lived. They measured the concentrations of NFκB and TNFα levels in the infliximab-treated patients and found that NFκB and TNFα were significantly higher in patients prior to relapse than in patients staying in remission. They concluded that these results underscore the important pathophysiological

role of TNFα secretion and NFκB activation in intestinal inflammation in CD. Again, these results demonstrate the correlation between TNFα levels and CD, and provide the basis for developing additional therapies that target TNFα for the treatment of CD.

Like many of the disorders described above, CD is an example of an immunoinflammatory disorder where, based on the results showing the relevance of TNFα in the pathogenesis of the disease, TNFα inhibitors were tested for therapeutic efficacy in the treatment of CD. One of the TNFα inhibitors, infliximab, was granted FDA approval for the treatment of CD in 1998.

Infliximab has been proven to be efficacious in the treatment of IBD including fistulating disease. The FDA approval of infliximab as maintenance therapy was based, in part, on data from the ACCENT I trial, a multi-center, randomized international trial of 545 patients with moderate-to-severely active CD who failed conventional therapies (e.g., 5-aminosalicylates, immunomodulators, and steroids). The trial continued through 54 weeks. The findings of the ACCENT I study demonstrated that at week 2, 57% of patients were in clinical response and at week 30, a significantly greater proportion of patients in the infliximab maintenance groups achieved clinical remission compared to patients in the placebo maintenance group. Additionally, a significantly greater proportion of patients in the infliximab maintenance groups were in clinical remission and were able to discontinue corticosteroid use compared to patients in the placebo maintenance group at week 54. The success of this trial contributed to FDA approval of infliximab for acute and maintenance therapy for CD.

Infliximab has also been approved for fistulizing CD based, in part, on the ACCENT II trial, which included a total of 296 patients with fistulizing CD in North America, Europe, and Israel. For this study, patients with either single or multiple draining enterocutaneous fistulas for at least three months were given a dose of infliximab at weeks 0, 2, and 6, and checked for a fistula response throughout the 54-week study.

The results of this study demonstrated that infliximab induced and maintained a fistula response, and reduced the number of hospitalizations and surgeries in the treated subjects.

In 2007, Abbott received FDA approval to market adalimumab for the treatment of moderate-to-severely active Crohn's disease. FDA approval was based on their success with the drug in three randomized, double-blind, placebo-controlled, multi-center trials: CLASSIC I, CHARM, and GAIN. In these trials, adalimumab demonstrated statistical significance in inducing and maintaining clinical remission in patients with moderate-to-severe Crohn's disease. These trials are summarized below.

CLASSIC I was a study of 299 patients with moderate-to-severe Crohn's disease, which showed that initiating treatment with adalimumab resulted in a statistically significant greater percentage of patients achieving clinical remission at four weeks compared to placebo.

CHARM was a 56-week trial of patients with moderately-to-severely active Crohn's disease. The 499 patients who demonstrated clinical response to adalimumab during a four-week open-label induction phase were randomized to receive either adalimumab or placebo. A statistically significantly greater percentage of those who continued on adalimumab maintained clinical remission through one year compared to placebo.

GAIN evaluated the efficacy of adalimumab in moderately-to-severely active Crohn's disease patients who had previously lost response or were unable to tolerate infliximab, a group of patients currently without effective treatment options. The results from the GAIN trial are not yet publicly available.

As with the disorders listed above, there are numerous additional studies in the literature that describe the treatment of CD with a TNFα inhibitor. Raza et al. (*Microscopy Res. Tech.* 50:229-235, 2000) demonstrated that with infliximab therapy, clinical improvements in CD become apparent within 5 days, and last for 10-12 weeks in most patients. Mpofu et al. (*Rheumatology* 44:271-272, 2005) compares the efficacy of

TNFα inhibitors in various inflammatory disorders and states that in CD there is a clear clinical benefit with infliximab treatment but not with etanercept. This discrepancy between infliximab and etanercept is noted throughout the literature (see, for example, Sandborn et al., *Gastroenterology* 121:1088-1094, 2001) and some of the proposed explanations include an ineffective dose, different capacities to penetrate the gut wall, and the ability of infliximab to lyse cells expressing surface TNFα contributing to the suppression of the inflammatory response. A summary of some additional clinical trials of TNFα inhibitors for the treatment of CD is provided in Ganesan et al. (*Curr. Op. Invest. Drugs* 3:1301-1306, 2002).

In sum, there is ample evidence in the literature demonstrating elevation of TNFα levels in patients with CD, particularly at the sites of mucosal inflammation. This evidence, when combined with the FDA approval of two TNFα inhibitors for treatment of CD, provides a sound basis for the use of compounds that inhibit TNFα production or activity for the treatment of CD. Accordingly, in view of the evidence provided demonstrating a role for TNFα inhibitors for the treatment of CD, Applicants submit that the synergistic effect of the claimed combination of a corticosteroid and an SSRI against TNFα secretion/production would reasonably be expected to translate into an effective treatment of CD. Applicants respectfully request that the enablement rejection, as it applies to CD, be withdrawn.

Ankylosing Spondylitis

In support of enablement of the claimed methods for treatment of ankylosing spondylitis (AS) in a subject, Applicants provide evidence that TNFα is elevated in AS and that inhibition of TNFα, through the use of TNFα inhibitiors, is an effective therapy against AS. Applicants point out that all three TNFα inhibitors have been FDA-approved for the treatment of AS.

There are several lines of evidence that support the role for TNFα in the pathogenesis of AS. One line of evidence is the demonstration, by several groups, that TNFα is over-expressed in the sacroiliac joints of patients with AS (Nanda et al., *Expert. Opin. Pharmacother.* 5:1175-1186, 2004; Gorman et al., *N. Eng. J. Med.* 346:1349-1356, 2002; Braun et al., *Ann. Rheum. Dis.* 61(Suppl. III):iii51-iii60, 2002). The second line of evidence comes from Crew et al. (*J. Interferon Cytokine Res.* 18:219-225, 1998), who generated transgenic mice that overexpressed a truncated mouse TNFα gene. They found that the mouse had an arthritic phenotype that closely resembled human AS and consisted of severe axial skeletal kyphosis and ankylosis. The mouse model of Crew et al. demonstrates, *in vivo,* the correlation between mouse TNFα overexpression and the development of AS.

The findings provide evidence under types 1 and 2 (above) that TNFα contributes to the pathogenesis of AS. Further, these findings were used to established the scientific rationale behind the development of clinical trials testing TNFα inhibitors for the treatment of AS. The end result of these trials was that all three inhibitors, namely, etanercept, infliximab, and most recently, adalimumab, have been approved by the FDA for the treatment of AS (Type 3, above). Some of the data from these clinical trials is summarized below.

The approval of etanercept was based on a randomized, double-blind, placebo-controlled study of 277 patients. After six months of twice-weekly treatments, 58% of patients who received etanercept showed significant improvement on a scale that measured pain, function, and inflammation compared to 23% who received a placebo.

The approval of infliximab for AS was based primarily on the 24-week results of the ASSERT trial, which demonstrated that AS patients treated with infliximab achieved significant improvement in signs and symptoms associated with their disorder, including reduced spinal pain and increased physical function. ASSERT was a randomized Phase III placebo-controlled, double-blind, 33-center trial conducted in North America and

Europe. The trial included 279 patients: 201 patients were treated with infliximab and 78 patients received placebo infusions.

In the infliximab group, 60% of patients achieved ASAS 20 (Assessment in Ankylosing Spondylitis Response Criteria, a composite score that includes spinal pain, inflammation, and functionality) compared with 18% of patients in the placebo group (p less than 0.001). Clinical benefit was observed in patients receiving infliximab as early as week two and was maintained over the 24-week study period. Patients receiving infliximab also showed significant improvements in individual measurements of disease activity, function, and mobility, as well as improvements in chest expansion and patient global assessment.

The approval of adalimumab for the treatment of patients with active AS was based, in part, on data from the ATLAS trial. ATLAS was a randomized, placebo-controlled, double-blind, Phase III study conducted in Europe and the United States. Results showed that adalimumab was successful in reducing pain and inflammation in patients with AS after 12 weeks of treatment. Other findings demonstrated significant improvement in measures of disease activity for many patients treated with adalimumab that were first observed at week two and maintained through 24 weeks. ATLAS also explored the impact of HUMIRA on enthesitis, a condition in AS characterized by inflammation of the ligaments that attach to the bone. At week 24, the mean change in the enthesitis symptom score showed significant reduction.

Other trials of TNFα inhibitors for the treatment of AS have been conducted and published. For example, Braun et al. (*Ann. Rheum. Dis.* 61(Suppl. III):iii51-iii60, 2002) describe the results of ten international open label and randomized, controlled studies (eight with infliximab and two with etanercept) and conclude that both TNFα inhibitors (infliximab and etanercept) are effective in treating patients with AS. Braun et al. (*Arthritis Res.* 4:307-321, 2002) and Sieper et al. (*Ann. Rheum. Dis.* 60:iii58-iii61, 2001) also summarize additional anti-TNFα therapy trials for the treatment of AS. The authors

of both articles conclude that these trials demonstrated that TNFα inhibitors produced significant improvement in the clinical measurements of AS. Nanda et al. (*supra*) also summarize the results of studies conducted using etanercept for the treatment of AS, where significantly more patients in the etanercept group met criteria for response as compared to those in the placebo group.

The proven clinical efficacy of the three recited TNFα inhibitors for the treatment of AS, as evidenced by the FDA approval of all three, supports the assertion that TNFα has a pathogenic role in AS and that TNFα inhibitors provide an effective therapeutic modality for the treatment of AS.

In summary, there is strong evidence in the literature demonstrating a role for TNFα in the pathogenesis of AS and all three recited TNFα inhibitors have been tested and approved by the FDA for the treatment of AS. Taken together, Applicants submit that these findings are sufficient to support the relationship between TNFα inhibition and the treatment of AS, and that given the synergistic effects of Applicants' combination of a corticosteroid and an SSRI against TNFα secretion/production, one skilled in the art would reasonably expect Applicants' combination to provide an effective treatment for AS. Applicants respectfully request that the enablement rejection, as it applies to AS, be withdrawn.

Multiple Sclerosis

In support of enablement of the claimed methods for treatment of multiple sclerosis (MS) in a subject, Applicants provide evidence that TNFα is elevated in MS and that this elevation correlates with the activity of the MS lesions.

TNFα is postulated to play a role in MS pathogenesis and the evidence for this includes the finding that TNFα is found in MS lesions in association with $CD3^+$ T cells, microglia, and astrocytes. Several articles have reported a correlation between TNFα levels in the serum or culture supernatants and the clinical course of MS (see, for

example, Khoury et al., *Neurology* 53:758, 1999, and references cited therein). Beck et al. (*Acta. Neurol. Scand.* 78:318-323, 1988) describe a longitudinal study of interferon and TNFα expression in twenty MS patients and a healthy control group using a whole blood mitogen stimulation assay. Their results demonstrated that prior to exacerbations, an increase in IFNγ and TNFα production were detected preceding clinical symptoms. These results led Beck et al. to conclude that IFNγ and TNFα may trigger exacerbations at a very early stage and that these cytokines may also play a role in maintaining disease. Similarly, Selmaj et al. (*J. Clin. Invest.* 87:949-954, 1991) identified TNF-α and lymphotoxin in acute and chronic active MS lesions but found that these markers were absent from chronic silent lesions. TNFα expression was found specifically in astrocytes and foamy macrophages in acute lesions indicating that the activation of inflammatory cells to express TNFα and lymphocytes occurs within the CNS. These studies demonstrate that expression of TNFα is found at the active lesions within MS and may play a role in the inflammatory responses in the active MS lesions.

In one seemingly contradictory report, Vladic et al. (*Cytokine* 20:86-89, 2002) showed that TNFα, assayed by monoclonal antibody-based ELISA could not serve as a marker of MS activity because it could not be detected in the cerebrospinal fluid (CSF), and was only measured in 20% of the sera samples. However, this finding by Vladic et al. may be explained by the findings of Beck et al. and Selmaj et al., described above, that the elevated levels of TNFα are found specifically in the active MS lesions or prior to exacerbation, and may not be detected in the sera of MS patients. The result of Vladic et al. may be further explained by the results of Sharief et al. (*N. Engl. J. Med.* 325:467-472, 1991) which describes the finding that high levels of TNFα were detected in the CSF of patients with chronic progressive MS and not in patients with stable MS. For this study, an ELISA was used to measure TNFα in CSF and serum in 32 patients with chronic progressive MS and 20 patients with stable MS. They found high levels of TNFα in the CSF of 53% of patients with chronic progressive MS, and the mean TNFα levels were

significantly higher in the CSF than in the corresponding serum samples in these patients. This lack of increase in the serum samples could explain why Vladic et al. did not detect TNFα in the sera of MS patients. Sharief et al. conclude from their studies that TNFα is likely released into the intrathecal compartment and that the elevated levels of TNFα in CSF correlates with the severity and progression of the disease. Sharief et al. further conclude that "the removal of TNFα or neutralization of its effect might be of benefit in patients with chronic progressive multiple sclerosis" (pg. 471, left column, 4th paragraph to right column, 1st paragraph).

Taken together, the data suggest that changes in the production of TNFα may occur before onset of neurological symptoms, and is likely to occur in acute or chronic MS lesions. As indicated by the excerpt from Sharief et al., such data provides a strong basis for the development and testing of TNFα inhibitors in the treatment of acute or chronic MS. Data from the use of TNFα inhibitors for the treatment of MS is not yet available.

In sum, Applicants have shown evidence from category 1 above that levels of TNFα are increased in the active MS lesions. Applicants submit that, as with these other disorders, the scientific evidence supports the relationship between inhibition of TNFα and treatment of MS, and that TNFα inhibitors may indeed prove to be an effective therapeutic for MS. Applicants submit that the synergistic effect demonstrated for the presently claimed combination would reasonably be expected to translate into an effective treatment for MS. Applicants respectfully request that the enablement rejection, as it applies to MS, be withdrawn.

Polymyalagia Rheumatica

In support of enablement of the claimed methods for treatment of polymyalagia rheumatica (PMR) in a subject, Applicants provide evidence that TNFα is associated with

PMR and that inhibition of TNFα, through the use of a TNFα inhibitior, is an effective therapy against PMR.

PMR is a chronic inflammatory condition that is characterized by aching and morning stiffness in the cervical region, shoulders, and pelvic girdles. TNFα is postulated to play a role in the pathogenesis of PRM, and evidence for its role is the increased incidence of TNFα microsatellite polymorphisms in patients with PMR relative to control subjects (Mattey et al., *Arth. Rheum.* 43:1749-1755, 2000). The postulated role of TNFα in the pathogenesis of PMR is further supported by preliminary clinical studies that demonstrate the effectiveness of TNFα inhibition on the treatment of PMR.

Migliore et al. (*Eur. Rev. Med. Pharmacol. Sci.* 9:373-378, 2005) performed a study of the affect of infliximab on seven patients having PMR. In the study, all seven patients were administered four infliximab infusions at a dose of 3 mg/kg at day 0, day 15, and at weeks 6 and 24 following the first infusion. Following the last infliximab infusion, methotrexate was introduced (7.5 to 10 mg per week) to maintain remission. The clinical outcome of the study was monitored by measuring the erythrocyte sedimentation ratio (ESR), C-reactive protein (CRP), glycaemia, and heamoglobin A_{1C} ration at weeks 0, 6, and 14. Patients enrolled in the study also reported in for a follow-up appointment at 7-9 months.

Migliore et al. report that all seven PMR patients receiving the four infliximab infusions showed a remission of clinical symptoms, and five of the seven treated patients showed normalization in the serological parameters ESR and CRP. The observed mean reductions in ESR and CRP values, at weeks 6 and 14, were statistically significant when compared to baseline values (p value less than 0.05). None of the five treated patients also having diabetes had an increase in haemogloblin A_{1C} ratio or glycemia, nor needed modification of their diabetes medications. After a mean 8-month follow-up, the symptoms and clinical features in these patients were still under control with weekly administration of methotrexate. None of the seven patients needed steroid therapy during

the follow-up period. The results of this preliminary study suggest that TNFα inhibition can be useful in the treatment of PMR patients.

In summary, there is evidence in the literature indicating a potential role for TNFα in the pathogenesis of PMR and a preliminary clinical study that demonstrates the effective treatment of PMR using a TNFα inhibitor. Taken together, Applicants submit that these findings are sufficient to support the relationship between TNFα inhibition and the treatment of PMR, and that given the synergistic effects of Applicants' combination of a corticosteroid and an SSRI against TNFα secretion/production, one skilled in the art would reasonably expect Applicants' combination to provide an effective treatment for PMR. Applicants respectfully request that the enablement rejection, as it applies to PMR, be withdrawn.

Giant Cell Arteritis

In support of enablement of the claimed methods for treatment of giant cell arteritis (GCA) in a subject, Applicants provide evidence that TNFα is associated with GCA and that inhibition of TNFα, through the use of a TNFα inhibitior, is an effective therapy against GCA.

GCA or temporal arteritis is an acute vasculitis characterized by destruction of arterial architecture, particularly the intima, media, and internal elastic lamina, following infiltration of the arterial wall by macrophages, giant cells, and lymphocytes. TNFα has been implicated for a role in the pathogenesis of GCA. Field et al. (*Rheumatol. Int.* 17:113-118, 1997) performed immunohistochemical assays on biopsies recovered from GCA patients. Field et al. discovered that TNFα protein was predominantly detected in the inflamed intima and media of GCA biopsy samples, on either side of the internal elastic lamina - the predominant site of tissue destruction in GCA. The data of Field et al. also demonstrate that TNFα was also primarily located in areas infiltrated by macrophages and giant cells, and that up to 60% of the cells in all areas of inflamed

arteries were immunopositive for TNFα protein. Field et al. also reported that more cells staining for TNFα were detected in the intima and media of inflamed vessels than control uninflamed arteries ($p < 0.003$ and $p < 0.001$, respectively).

In addition to the immunohistochemical studies reported in Field et al., Mattey et al. (*supra*) have shown that there is an increased incidence of TNFα microsatellite polymorphisms in patients with GCA relative to control subjects. The combined data of Field et al. and Mattey et al. indicate a role for TNFα in the pathogenesis of GCA. This role for TNFα is further supported by three preliminary clinical studies that show a positive effect of TNFα inhibition on the clinical outcome of patients with GCA.

Cantini et al. (*Arth. Rheum.* 44:2933-2935, 2001) reports the treatment of four GCA patients with infliximab. Each of the four treated patients in the study had previously undergone long courses of corticosteroid treatment without achieving remission. The treated patients were administered three intravenous infusions of infliximab (3 mg/kg) at weeks 0, 2, and 6, and 5 mg/day prednisone during the first two weeks. The administration of the prednisone was ceased if remission was obtained after the second infusion of infliximab. The third infusion of infliximab was administered only if a patient had achieved clinical remission after the second infusion. The disease parameters that were measured in this study were ESR and CRP levels, as well as physical symptoms, including systemic symptoms, cranial symptoms, and articular symptoms.

Three of the four patients in this study demonstrated a complete response to the infliximab therapy, with both clinical and humoral remission after the second infusion. The remission in the three patients continued after the third infusion and during the follow-up period without any treatment (after 5-6 months from the third infliximab infusion).

In a second published case study, Ahmed et al. (*Clin. Rheumatol.* 26:1353-1355, 2007) administered adalimumab to a patient having resistant GCA. The patient

demonstrated an alleviation of symptoms following treatment with adalimumab and remained asymptomatic up to six months after starting adalimumab.

In a third placebo-controlled study reported by Martinez-Taboada et al. (*Ann. Rheum. Dis.* 67:625-630, 2008), eight GCA patients were administered etanercept and nine GCA patients received a placebo over 1 year, with corticosteroids that were reduced according to a predefined schedule. After 1 year in the study, 50% of the patients in the etanercept group and 22.2% in the placebo group were able to control the disease without corticosteroid therapy.

In summary, there is evidence in the literature demonstrating a potential role for TNFα in the pathogenesis of GCA and three preliminary clinical studies that demonstrate the effective treatment of GCA using TNFα inhibitors. Taken together, Applicants submit that these findings are sufficient to support the relationship between TNFα inhibition and the effective treatment of GCA, and that given the synergistic effects of Applicants' combination of a corticosteroid and an SSRI against TNFα secretion/production, one skilled in the art would reasonably expect Applicants' combination to provide an effective treatment for GCA. Applicants respectfully request that the enablement rejection, as it applies to GCA, be withdrawn.

Summary

Applicants have provided evidence that establishes the relationship between the inhibition of TNFα and the treatment of each of the immunoinflammatory disorders recited in independent claim 26. The evidence provided includes the demonstration or indication of an elevated level of TNFα that correlates with the immunomodulatory disorder, demonstration of the disorder or an etiologically similar disorder in transgenic animals overexpressing TNFα, and amelioration or prevention of the disorder through the use of TNFα inhibitors.

Importantly, for all but three of the indications described above, the use of TNFα inhibitors has not only been shown to be effective, but the use of TNFα inhibitors *has been approved by the FDA* for the treatment of the disorder. Given the rigorous evaluation of the safety and efficacy data by a team of physicians, statisticians, chemists, pharmacists and other scientists at the FDA, approval of a TNFα inhibitor for the treatment of a particular disorder provides an almost indisputable correlation between inhibition of TNFα and treatment of the disorder.

In view of the evidence provided above, Applicants respectfully submit that the synergistic effects proven by Applicants for the claimed combination of a corticosteroid and an SSRI against TNFα secretion/production would provide a skilled artisan sufficient basis for a reasonable expectation that the claimed combination would provide effective treatment for each of the immunoinflammatory disorders of the amended claims. Applicants respectfully request that the enablement rejection be withdrawn.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are also rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the term "analog." As stated above, Applicants have amended claims 25 and 26 to remove the term "analog" and therefore, this rejection may be withdrawn.

Rejections under 35 U.S.C. § 103(a)

Rejection over Siegel

Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Siegel. As the basis for this rejection, the Office states that Siegel teaches "a method of decreasing proinflammatory cytokine secretion...in patients with autoimmune disease...by use of an immunosuppressive agent, including the glucocorticoid prednisolone in combination with the selective serotonin

reuptake inhibitor (SSRI) paroxetine..." (Office Action, pg. 9) and that "it would have been obvious to have selected various combinations of various disclosed ingredients from within a prior art disclosure, to arrive [at] compositions 'yielding no more than one would expect from such an arrangement'" (Office Action, pg. 10). Applicants respectfully traverse this rejection.

The Office cites Siegel for teaching the combination of an SSRI and a corticosteroid. Siegel teaches the combination of an immunosuppressive agent from a list of 14 different agents (e.g., glucocorticoid; column 3, lines 49-56) and an additional agent from a list of 29 agents (e.g., a selective serotonin reuptake inhibitor (SSRI), fluoxetine, paroxetine, and sertraline; column 3, line 58, to column 4, line 3) for the treatment of narcolepsy and cataplexy. Applicants submit that the Office has failed to provide a reason why one skilled in the art would choose the combination of an SSRI and a glucocorticoid from the 406 different combinations (14 x 29 = 406) disclosed by Siegel, and, for this reason, a *prima facie* case of obviousness has not been made.

The Supreme Court recently commented on the standard for obviousness rejections:

> Although common sense directs one to look with care at a patent application that claims as innovation the combination of two known devices according to their established functions, it can be important to *identify a reason* that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does. KSR Int'l Co. v. Teleflex Inc. 550 U.S. _____, 82 USPQ2d 1385 (2007) (emphasis added).

Applicants submit that the Office has not provided a reason why a person skilled in the art would arrive at the Applicants' invention in view of the large number of potential combinations disclosed in Siegel.

Moreover, as mentioned above, Applicants have discovered that the combination of an SSRI and a corticosteroid has an unexpected, synergistic effect on decreasing proinflammatory cytokine secretion/production (see, Tables 15-22 of the specification).

The Supreme Court's recent comments on *United States v. Adams* (383 U.S. 39 (1966)) in *KSR International Co. v. Teleflex Inc.* (550 U.S._____, 82 USPQ2d 1385 (2007), page 13) support a finding of nonobviousness in view of an unexpected result:

> The fact that the elements worked together in an unexpected and fruitful manner supported the conclusion that Adams's design was *not obvious* to those skilled in the art. (Emphasis added).

In addition, the USPTO's Examination Guidelines for Determining Obviousness Under 35 103 In View of the Supreme Court Decision in *KSR International Co. v. Teleflex Inc.* (Federal Register Vol. 72, No. 195, page 57534) instructs:

> [I]n the case of a claim to a combination, applicants may submit evidence or argument to demonstrate that:
>
> (1) one of ordinary skill in the art could not have combined the claimed elements by known methods (e.g., due to technological difficulties);
>
> (2) the elements in combination do not merely perform the function that each element performs separately; or
>
> (3) the results of the claimed combination were *unexpected.* (Emphasis added).

As noted above, the specification provides data (see, Tables 15-22) that demonstrate the unexpected effect of the combination of an SSRI and a corticosteroid on decreasing proinflammatory cytokine secretion/production. These data are summarized in the enclosed Exhibit 1, where it is clearly shown that the combined effect of an SSRI with a corticosteroid results in an effect that is greater than the sum of the effects of the SSRI and corticosteroid when administered alone.

As Siegel does not teach the synergistic effect of the combination of an SSRI and a corticosteroid on decreasing proinflammatory cytokine secretion or production, Applicants therefore, respectfully request that the rejection in view of Siegel under 35 U.S.C. § 103(a) be withdrawn.

Rejection over Shi

Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are also rejected under 35 U.S.C. § 103(a) as being unpatentable over Shi. As the basis for this rejection, the Office states that Shi teaches the "treatment of various diseases by polypeptides of the application..., in combination with other agents, such as prednisolone, methotrexate, HYDELTRASOL.TM (prenisolone)...and paroxetine..." (Office Action, pg. 11) and that "it would have been obvious to have selected various combinations of various disclosed ingredients from within a prior art disclosure, to arrive [at] compositions 'yielding no more than one would expect from such an arrangement'" (Office Action, pg. 12). Applicants respectfully traverse this rejection.

The Office cites Shi for teaching the combination of an SSRI and a corticosteroid. Shi teaches the combination of one of their disclosed polypeptides with an immunosuppressive agent from a list of 24 different agents (e.g., prednisolone, glucocorticoids, prednisone, and prednisolone; pg. 124, paragraph 1101) or with a psychiatric drug from a list of 55 agents (e.g., citalopram, fluvoxamine, fluoxetine, paroxetine, and sertraline; pg. 129, paragraph 1130) for the treatment of a variety of disorders. Applicants submit that the Office has failed to provide a reason why one skilled in the art would choose the combination of an SSRI and a glucocorticoid from the 1,320 different combinations (24 x 55 = 1,320) disclosed by Shi, and, for this reason, a *prima facie* case of obviousness has not been made.

Moreover, Applicants have discovered that the combination of an SSRI and a corticosteroid has an unexpected, synergistic effect on decreasing proinflammatory cytokine secretion/production (see, Tables 15-22 of the specification, and Exhibit 1). As noted above, the decision in *KSR International Co. v. Teleflex Inc.* (550 U.S._____, 82 USPQ2d 1385 (2007), page 13) supports a finding of nonobviousness in view of an unexpected result. As Shi does not teach the synergistic effect of the combination of an SSRI and a corticosteroid on decreasing proinflammatory cytokine secretion or

production, Applicants therefore, respectfully request that the rejection in view of Shi under 35 U.S.C. § 103(a) be withdrawn.

Rejection for Double Patenting

Claims 25-28, 30, 45-50, 55-58, 61-65, and 81 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 22-41, 49-53, and 70 of copending Application Serial No. 10/670,488. Applicants wish to hold this rejection in abeyance until indications of allowable subject matter in this application have been received.

CONCLUSION

Applicants submit that the application is now in condition for allowance and such action is hereby respectfully requested.

Transmitted herewith is a Petition to extend the period for replying to the Office Action for three months, to and including September 22, 2008, as September 20, 2008 is a Saturday, and payment of the required extension fee.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 9/22/08

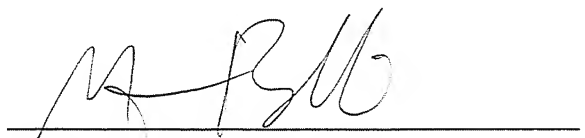

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Exhibit 1

Support in Specification	SSRI	SSRI Dose	Corticosteroid	Corticosteroid Dose	% Decrease SSRI Alone	% Decrease Corticosteroid Alone	Expected % Decrease of Combination	Actual % Decrease of Combination
Table 15	paroxetine	0.375 μM	prednisolone	0.025 μM	1.4	16.5	17.9	**22.4**
Table 16	fluoxetine	7.23 μM	prednisolone	0.006 μM	29.6	7.0	36.6	**42.4**
Table 17	fluoxetine	0.009 μM	budesonide	0.009 μM	7.9	5.3	13.2	**43.6**
Table 18	paroxetine	3.0 μM	dexamethasone	0.0063 μM	9.9	26.7	36.6	**42.0**
Table 19	fluoxetine	0.036 μM	dexamethasone	0.0024 μM	22.7	0.25	23.0	**35.0**
Table 20	fluoxetine	1.80 μM	prednisolone	0.0160 μM	5.5	10.5	16.0	**19.4**
Table 21	paroxetine	3.30 μM	prednisolone	0.016 μM	29.1	12.9	42.0	**50.3**
Table 22	sertraline	4.0 μM	prednisolone	0.0160 μM	19.4	6.3	25.7	**29.0**

EXHIBIT 2

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ANTI-TNFα THERAPY OF RHEUMATOID ARTHRITIS: What Have We Learned?

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Key Words TNFα, cytokines, anti-TNFα antibodies and inhibitors, rheumatoid arthritis, immunotherapy

■ **Abstract** Rheumatoid arthritis (RA), a systemic disease, is characterized by a chronic inflammatory reaction in the synovium of joints and is associated with degeneration of cartilage and erosion of juxta-articular bone. Many pro-inflammatory cytokines including TNFα, chemokines, and growth factors are expressed in diseased joints. The rationale that TNFα played a central role in regulating these molecules, and their pathophysiological potential, was initially provided by the demonstration that anti-TNFα antibodies added to in vitro cultures of a representative population of cells derived from diseased joints inhibited the spontaneous production of IL-1 and other pro-inflammatory cytokines. Systemic administration of anti-TNFα antibody or sTNFR fusion protein to mouse models of RA was shown to be anti-inflammatory and joint protective. Clinical investigations in which the activcity of TNFα in RA patients was blocked with intravenously administered infliximab, a chimeric anti-TNFα monoclonal antibody (mAB), has provided evidence that TNF regulates IL-6, IL-8, MCP-1, and VEGF production, recruitment of immune and inflammatory cells into joints, angiogenesis, and reduction of blood levels of matrix metalloproteinases-1 and -3. Randomized, placebo-controlled, multi-center clinical trials of human TNFα inhibitors have demonstrated their consistent and remarkable efficacy in controlling signs and symptoms, with a favorable safety profile, in approximately two thirds of patients for up to 2 years, and their ability to retard joint damage. Infliximab (a mAB), and etanercept (a sTNF-R-Fc fusion protein) have been approved by regulatory authorities in the United States and Europe for treating RA, and they represent a significant new addition to available therapeutic options.

INTRODUCTION

Rheumatoid arthritis is one of the commonest human autoimmune diseases, with a prevalence of 1%. There is a genetic predisposition, with the clearly defined involvement of HLA of DR4/DR1 (1). A shared epitope, a sequence between amino acid positions 70 and 74 of the DRB chain that is shared by DR4/DR1 susceptible

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haplotypes, has been identified (2). This implicates $CD4^+$ T cell recognition at some stage of the disease process (3). Concordance in twin studies is low, ranging from 35% (4) to 15% (5), indicating the role of nongenetic factors.

The clinical features are mostly due to inflammation and eventual damage to synovial joints of hands, feet, wrists, knees, hips, etc. In more severe cases, there is extra articular disease, and survival is impaired (6, 7).

The synovitis involves a massive leucocytic infiltrate chiefly consisting of macrophages, T lymphocytes, and plasma cells, and this is associated with augmented angiogenesis. Where the synovium abuts the cartilage and bone is the site of major joint damage. Details of the features of rheumatoid arthritis have been documented in detail elsewhere (8, 9).

Pathophysiology of TNFα

Despite the discovery of TNFα bioactivity in the 1970s by Old and colleagues (10, 11) and its cloning (12–14) under different names in the 1980s (such as cachectin by Cerami and colleagues), the pathophysiological effects of TNFα and its sister molecule lymphotoxin (15, 16) are still incompletely understood. It is clear that many of the in vitro experiments, performed with abundant recombinant TNFα (like many other cytokines) at supraphysiological doses, does not represent what happens in vivo at the much more limiting physiological doses, and in the presence of inhibitors and regulatory pathways. Based on in vitro analysis, it appears that TNFα, LTα, IL-1α and β have almost identical function, and GM-CSF exerts almost the same effects. Hence the concept of cytokine redundancy was conceived (17, 18), which suggested that many cytokines had almost the same properties, but the reasons for this cytokine redundancy were never clear. However, experiments in vivo using neutralizing antibodies and especially targeted mutations or gene knockouts have shown that cytokine redundancy is much less obvious in vivo.

The major source of TNFα is the cells of the monocyte/macrophage lineage, with T lymphocytes, neutrophils, mast cells, and endothelium also contributing under different circumstances. All potentially noxious stimuli, ranging from the physical (ultraviolet light, X-radiation, heat) to the chemical and immunological, can rapidly induce TNFα production and release (14, 19). In vivo TNFα is the most rapidly produced pro-inflammatory cytokine, with serum levels detectable in mice in 30 min (20). Probably the earliest TNFα comes from preformed stores by cleavage of membrane TNFα on macrophages, neutrophils, and activated T cells by TNFα converting enzyme (TACE/ADAM 17) (21), and release of cytoplasmic granules from mast cells and eosinophils. Subsequent release of TNFα is due to new synthesis, chiefly in macrophages and T lymphocytes.

If the rapid release of TNFα at times of stress is blocked, the expression of other pro-inflammatory cytokines, such as IL-1 and IL-6, is reduced (22). This and analogous in vitro data (23) suggest that TNFα in vivo coordinates the cytokine response to injury and acts as a fire alarm. The induction by TNFα of multiple chemokines and adhesion molecules (24, 25) is of major importance in rapidly attracting

immune and inflammatory leukocytes to the site of injury and TNFα release. TNFα also acutely upregulates the function of the immune system (26, 27), but following prolonged exposure to an excess of TNFα, it is immunosuppressive (28).

More details of the multiple functions of TNFα can be found in the references (13, 14, 19).

Cytokine Network

The description of cytokines as chemical entities got off to a slow start. It seemed initially that many of the multiple biological activities, described for cell-free supernatants termed cytokines, were due to a small set of proteins. Thus interleukin (IL)-1 was independently discovered as lymphocyte activating factor, connective tissue degradative catabolin, and endogenous pyrogen (reviewed in 29). However, over 150 cytokines have been identified and cloned (30), and by genome sequencing many more cytokine-like molecules of unknown function have been uncovered. Many recently described cytokines are not secreted by cells but act by cell-to-cell contact, thus confounding the initial definition of cytokines as secreted proteins acting as short range intercellular messenger molecules. For an updated description of the current status of cytokines, see (30).

Cytokines are never expressed singly by a cell or tissue. Instead, an activated cell (e.g. a macrophage) produces a wide spectrum of cytokines. Similarly all cells express receptors for many, but not all, cytokines. Unlike hormones that are expressed constitutively, most cytokines are expressed transiently after an inducing stimulus. One of the most potent signals for inducing cytokines are other cytokines, and so the concept has arisen of a cytokine network in which cytokines induce or inhibit each other (17, 31, 32). This accounts, in part, for the complexity of cytokine expression found at any diseased tissue site such as the rheumatoid synovium. How this complex mixture of molecules, interacting with multiple cells, is regulated is currently only partly understood, but it is becoming evident that dysregulation of the cytokine network contributes in a major way to the pathogenesis and pathology of rheumatoid arthritis (31–37).

Key aspects of the cytokine network in health include the transient expression of cytokine genes in contrast to the constitutive but regulated expression of cytokine receptors. However, cytokine receptors also form part of the regulatory system as they are downregulated by ligand interaction and most importantly are cleaved by metalloproteinase enzymes (21, 38) to yield soluble cytokine receptors (39–41). Many, probably most of these soluble receptors [e.g. soluble IL-2 receptor (sIL-2R), soluble TNF-receptor (sTNF-R)] are still capable of binding the cytokine and hence act as inhibitors by competing with membrane-bound signaling receptors. Cleavage of receptors also reduces their cell surface density, and since cytokine receptors usually need to be aggregated by their ligand to generate a signal, the signaling capacity of the cell is inhibited.

Concomitantly, soon after the release of pro-inflammatory cytokines, other cytokines with chiefly anti-inflammatory properties are released that act to limit

the duration and extent of the pro-inflammatory effect. These inhibitory cytokines include IL-10, TGFβ, IL-11, and IL-1 receptor antagonist (IL-1Ra) (35, 42–46).

The relatively easily accessible rheumatoid synovium has become the most extensively analyzed tissue site of a local immune and inflammatory reaction. Hence much is known about synovial cytokine expression. This has been studied in a variety of ways, for example, on fresh ex vivo tissue by immunohistology (47) or in situ hybridization (48), by examining its waste products found in the synovial fluid (49, 50), and also by short-term culture of synovial membrane cells in the absence of extrinsic stimulation (23). The latter technique permits the quantitative analysis of released proteins and their inhibitors, and it is the procedure we have studied most extensively. Using such cultures, we and others have documented expression of a great many cytokines in this tissue. The data has demonstrated that there is up-regulation not only of pro-inflammatory cytokines but also of the anti-inflammatory cytokines and cytokine inhibitors, including soluble receptors (23, 47–57).

Our attempts to understand how this plethora of mediators was regulated in the absence of extrinsic stimulation (23) led us to use antibodies to cytokines as pharmacological antagonists. This revealed the profound effects of anti-TNFα antibody in reducing the production of IL-1 in rheumatoid synovial cultures and led us to the current understanding that TNFα was of major importance in regulating the activities of other cytokines (23, 33, 34). A grossly simplified summary of the cytokine network in RA is shown in Figure 1, which does not include the multiple cell interactions or the effects of cell recruitment and apoptosis that are most likely involved under physiological conditions at sites of inflammation.

Cytokine Expression and Regulation in RA

A summary of the many potentially important cytokines found in RA synovium is shown in Table 1. It shows that cytokines of essentially all classes are found with multiple activities, such as those with pro-inflammatory and anti-inflammatory

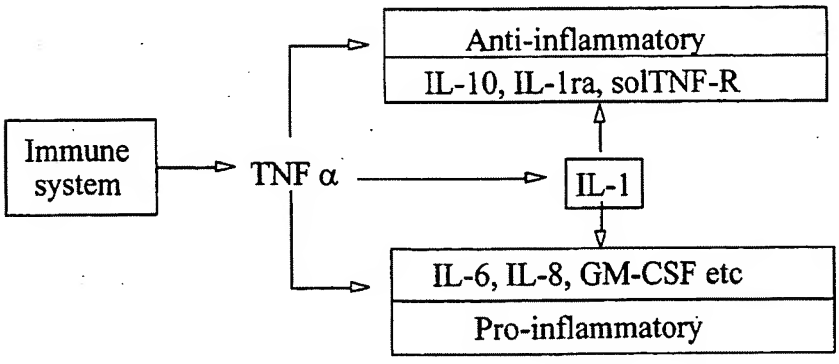

Figure 1 Cytokine cascade in rheumatoid arthritis. Modified from Feldmann, Elliott, Woody and Maini (1997) *Advances in Immunology* 64:283–350.

TABLE 1 Cytokine expression in rheumatoid arthritis

Cytokine	Expression	
	mRNA	protein
Pro-inflammatory		
IL-1α,β (interleukin 1)	+[a]	+
TNF (tumor necrosis factor alpha)	+	+
LT (lympotoxin)	+	±
IL-6 (interleukin 6)	+	+
GM-CSF (granulocyte macrophage colony stimulating factor)	+	+
M-CSF (macrophage colony stimulating factor)	+	+
LIF (leucocyte inhibitory factor)	+	+
Oncostatin M (oncostatin M)	+	+
IL-2 (interleukin 2)	+	±
IL-3 (interleukin 3)	−	−
IL-7 (interleukin 7)	?	?
IL-9 (interleukin 9)	?	?
IL-12 (interleukin 12)	+	+
IL-15 (interleukin 15)	+	+
IFN α,β (interferon alpha/beta)	+	+
IFN γ (interferon gamma)	+	±
IL-17 (interleukin 17)	+	+
IL-18 (interleukin 18)	+	+
Immunoregulatory		
IL-4 (interleukin 4)	±	−
IL-10 (interleukin 10)	+	+
IL-11 (interleukin 11)	+	+
IL-13 (interleukin 13)	+	+
TGF β (transforming growth factor beta)	+	+
Chemokines		
IL-8 (interleukin 8)	+	+
Gro α (melanoma growth stimulating activity)	+	+
MIP-1 (macrophage inflammatory protein)	+	+
MCP-1 (monocyte chemoattractant protein)	+	+
ENA-78 (epithelial neutrophil activating peptide 78)	+	+
RANTES (regulated upon activatioin T cell expressed & secreted)	+	+
Mitogens		
FGF (Fibroblast growth factor)	+	+
PDGF (Platelet-derived growth factor)	+	+
VEGF (vascular endothelial growth factor)	+	+

[a]Cytokines expressed in rheumatoid synovial tissue. + present, − absent

Modified after Feldmann, Brennan & Maini (1996), *Annual Review of Immunology* 14:397–440.

action, growth factors, interferons, etc. A major exception is IL-4 because the presence of this cytokine is very rarely reported in RA synovium. This could be of pathophysiological relevance, highlighting both the Th1 dominance of the immune-inflammatory process and the relative lack of its anti-inflammatory counter effect. In contrast other anti-inflammatory cytokines are upregulated and hence relatively abundant, including IL-10, IL-11, and TGFβ1. The latter are active in RA synovium, as neutralizing of IL-10 or IL-11, for example, markedly upregulates TNFα production (58, 59).

The complexity of cytokine expression in synovium obscures the role that any single cytokine might play in pathogenesis of disease. To this complexity, as noted by immunohistology, must be added microheterogeneity, in that cytokine expression varies from one region to another and is also different at the sites of cartilage and bone damage where the synovial membrane abuts on cartilage or bone, a tissue termed pannus (60, 61).

Despite this local heterogeneity, lost when the synovial tissue is dissociated, the regulation of rheumatoid cytokine expression studied in in vitro cultures was instrumental in providing the initial findings leading to a rationale for anti-TNFα therapy in rheumatoid arthritis. Many of the observations made in these rheumatoid synovial cultures have been verified to occur in vivo by analyses of clinical trials of anti-TNFα in rheumatoid patients (see below).

Cytokine expression in rheumatoid tissue has been extensively reviewed in recent years and hence is not documented in detail here. For comprehensive review see (33, 37).

Rationale for Anti-TNFα Therapy in RA

It is a misconception to think that TNFα was an obvious therapeutic target in the early 1990s since it is pro-inflammatory and present in synovium. The same could be said for IL-1, IL-6, GM-CSF, IL-8, and so on. The plethora of possible cytokine therapeutic targets and the concern about cytokine redundancy led some workers in the field to consider cytokines to be poor therapeutic targets. The prevailing view in the early 1990s was that blocking any one pro-inflammatory mediator in isolation would not be beneficial as those remaining would drive the biological processes.

The first clue that TNFα might be a good therapeutic target has already been alluded to, namely the effects of anti-TNFα antibody on cultures of dissociated rheumatoid synovial membranes (23). These cultures provide a good model of certain aspects of the disease as the cells in these suspensions, namely 30% T, 30%–40% macrophages, and fewer endothelial cells, fibroblasts, dendritic cells, plasma cells, B lymphocytes, etc, reaggregate in vitro and reproduce, in the absence of extrinsic stimulation, the molecules that are produced in vivo – e.g. TNFα, IL-1, GM-CSF, IL-6, VEGF, MMPs, PGE2, etc.

Addition of anti-TNFα antibody, but not the closely related anti-LTα antibody, inhibited production of IL-1 bioactivity (23). This prompted studies of other effects

TABLE 2 Rationale for anti-TNFα therapy

1. TNFα dependence of pro-inflammatory cytokines in rheumatoid synovial cultures
2. Anti TNFα ameliorates inflammation and joint destruction in murine collagen induced arthritis
3. Upregulated TNFα/TNF-R in joints in inflamed synovium and the destructive pannus-cartilage junction

of anti-TNFα in rheumatoid synovial cultures in vitro; downregulation of synovial GM-CSF (62), IL-6, IL-8 was noted (33). Hence blocking a single pro-inflammatory cytokine led to the diminution in production of other pro-inflammatory cytokines with closely related action in the diseased tissue (e.g. IL-1, GM-CSF, IL-6) and suggested that cytokine redundancy in this diseased tissue may not be a major problem.

As it is possible to artefactually induce pro-inflammatory cytokines rapidly in vitro, for example with LPS, which contaminates serum and glassware, the verification by immunohistologic approaches of upregulated TNF/TNF-receptor expression (not involving tissue culture) on freshly frozen tissue (47, 63) was a valuable confirmation that TNF/TNF-R interactions might be important in vivo. The findings also contributed to the emerging concept that TNFα might be a good therapeutic target in RA.

Whereas there are often significant differences between animal models of disease and the authentic human disease (64, 65), there are benefits of using animal models because physiological aspects of disease, e.g. cell trafficking, cannot be mimicked in vitro. Collagen-type II induced arthritis, which has many resemblances to RA but also some differences, was the model initially used to test whether TNFα blockade was beneficial in vivo. Several groups performed similar studies simultaneous with anti-TNFα agent and reported their results in 1992/1993 (66–69). All of them demonstrated a clear benefit of TNFα inhibition by antibodies or TNF-R Fc fusion proteins on collagen induced arthritis, most notably even if the treatment was begun after disease onset.

A summary of the rationale for anti-TNFα therapy of RA is presented in Table 2.

INHIBITORS OF TNFα IN CURRENT CLINICAL USE

At present (June 2000), the only drugs that are in clinical practice or in clinical trials to block TNFα are biologicals, protein-based drugs, either antibody to TNFα or based on TNFα receptors (e.g. linked to Fc dimers). These agents have the major advantage of specificity (70) but have significant disadvantages, including the need for repeated injection and their relative high cost compared to small organic chemical drugs (71).

Monoclonal Antibodies

Chimeric Monoclonal Anti-TNFα, Infliximab (Remicade™) This antibody was chimerized and is a mouse Fv, human IgG1, κ antibody of high affinity and neutralizing capacity with the potential for effector functions on human cells (72). This was the first anti-TNFα antibody used for therapy of RA (73) and is now approved for this indication and for Crohn's disease in the United States and more recently in Europe for both these indications.

Human Monoclonal Anti-TNFα Antibodies Subsequent to the use of infliximab in clinical trials of RA, other anti-TNFα antibodies and fusion proteins, which had been developed for use in sepsis, were diverted for use in RA. The first of these was a humanized, complementarity determining region (CDR) grafted monoclonal antibody, CDP571, developed by Celltech (74). Subsequently, a human antibody, D2E7, produced by phage display by Cambridge Antibody Technology, has entered clinical development with BASF/Knoll (75). The newest entrant to the anti-TNFα antibody field is a PEG-linked Fab fragment, CDP870, from Celltech, which can be produced in E. coli (76).

TNF Receptor Based Biologicals

As the TNF receptors possess a high affinity for TNFα, these molecules in their soluble form are also potential TNFα inhibitors. The cleaved soluble p55 and p75 TNF receptors are present in body fluids at ng/ml concentrations (39–41), act as physiological inhibitors, and have been engineered as pharmaceutical inhibitors, as described below.

p75 TNF-R Fc Fusion Protein, Etanercept (Enbrel™) As TNFα is a trimer, a TNF-R dimer would more effectively compete with binding of TNFα to the membrane receptors than a mononomer and prevent cell signaling. Engineered p75 TNFR dimers linked to Fc portion of IgG were shown to be effective inhibitors, first in animal models (77) and subsequently in clinical trials.

p55 TNF-R Fc Fusion Protein, Lenercept This is a similar dimer based on p55 TNF-R, but while it was effective in animal models (78), it was less successful in the clinic than etanercept or infliximal and has been abandoned.

Pegylated Truncated p55 TNF-R A truncated p55 TNF-R was produced in order to overcome the immunogenicity of a dimeric pegylated full-length p55 TNF-R. It is PEG-linked to prolong its circulating half-life (79).

CLINICAL EFFICACY OF ANTI-TNFα BIOLOGICALS

Since clinical trials of anti-TNFα biologicals in RA began in 1992, the results have been very consistent, with all the TNFα inhibitors tested being efficacious

TABLE 3 Anti-TNF agents in clinical trials in rheumatoid arthritis

Name	Composition	Manufacturer
Monoclonal antibodies		
Infliximab, Remicade™	Chimeric (mouse × human) mAb	Centocor, USA
CDP571	Humanized murine CDR3 engrafted mAb	Celltech, UK
D2E7	Human mAb	Cambridge Antibody Technology/BASF
Soluble TNFR:Fc (IgG) Fusion Proteins		
Etanercept, Enbrel™	p75TNFR:Fc	Immunex/American Home Products
Lenercept	p55TNFR:Fc	Roche, Switzerland

(Table 3). In the absence of direct comparisons, it is not possible to conclude whether apparent differences in outcome measurement between these agents are due to pharmacological effects or clinical heterogeneity of patient populations. However, the concept of TNFα as a major therapeutic target in RA has been amply validated, and two of these agents, etanercept and infliximab, have been approved for treatment of rheumatoid arthritis by the FDA and the European agency. At the time of writing (June 2000), almost 100,000 rheumatoid patients have been treated with one or other of these agents.

Clinical Efficacy of Anti-TNFα, Infliximab

Infliximab was the first anti-TNFα agent to be used for the treatment of RA and is the most intensively investigated in clinical pharmacological studies. The first Phase I/II study was an open (nonplacebo controlled) trial of infliximab in long-standing active RA patients who had failed all prior therapy; it was initiated in May 1992. A high dose of anti-TNFα antibody was given (20 mg/kg over 2 weeks in either 2 or 4 infusions) (73) as animal model studies had demonstrated that efficacy required a dose in this range (67).

The clinical results were notable, with patients reporting alleviation of symptoms such as pain, morning stiffness, tiredness, and lethargy within hours; a reduction in the numbers of swollen joints and tender joints was observed by 2 to 4 weeks. While all 20 patients in this trial benefited to a variable degree, the response was temporary, lasting from 8 to 22 weeks (73). A subset of these patients was re-treated (with 10 mg/kg) for a further 3 cycles of infusions with infliximab; each followed with a renewed response of a similar magnitude and limited duration (80). These highly encouraging results laid the foundation for the concept that repeated long-term treatment with anti-TNFα was possible for chronic diseases such as RA.

A double-blind, randomized, placebo controlled clinical trial quickly followed and established efficacy of infliximab in controlling signs and symptoms of RA.

Seventy-three patients with active RA despite previous anti-rheumatic therapy were given a single intravenous infusion of infliximab at either high (10 mg/kg) or low (1 mg/kg) doses. The end point of this study was response to therapy evaluated at the end of four weeks by the Paulus criteria, a composite index requiring improvements in at least four out of six variables, including tender and swollen joint counts, duration of morning stiffness and reduction in ESR or C-reactive protein (CRP), and the patient's and physician's global assessment of disease activity.

The results of this intention-to-treat analysis were clear cut, in that 2/24 (8%) patients given placebo infusion met these criteria, compared with 11 out of 25 (44%) at the low dose and 19 of 24 (79%) at the high dose of infliximab. All patients were followed to relapse; the median duration of response at 1 mg/kg was found to last 3 weeks and at 10 mg/kg lasted 8 weeks (81). The degree of improvement was high, with a 60%–70% reduction in measures of disease activity such as tender or swollen joint counts and CRP. The results of this trial not only provided a formal proof of concept (73), they also stimulated initiation of clinical trials with other TNFα inhibitors, e.g. lenercept, etanercept, CDP571, which had been developed for use in sepsis.

As established RA is a chronic disease, the aim of the next most important clinical trial was to demonstrate that multiple doses of anti-TNFα could be administered safely over a prolonged period without any loss of efficacy. Thus, a longer term trial was initiated involving patients with active disease despite methotrexate (MTX) therapy. Five infusions of placebo or infliximab at three dose levels were administered, either with a continuing fixed low dose of methotrexate, or without methotrexate over a 14-week period, with a final assessment at 6 months (82). Since MTX in low doses once a week has become established as one of the more durable and effective therapies for RA, patients whose disease activity persisted despite this drug were recruited in this randomized placebo trial. The enhanced efficacy of anti-TNFα therapy by anti-CD4 antibody in collagen induced arthritis provided the rationale for the combination of infliximab and MTX which also has anti-T cell activity (83).

A total of 101 patients with longstanding active disease were stabilized on MTX 7.5 mg weekly and allocated to one of seven groups. A control group received placebo infusions (plus MTX), three groups received infliximab at 1, 3, or 10 mg/kg of body weight plus MTX, and another three groups at 1, 3, or 10 mg infliximab plus placebo tablets instead of MTX. The response was assessed by the Paulus criteria at baseline and over the next 6 months.

In the absence of MTX, there was a very clear dose response. Infliximab at a dose of 1 mg/kg yielded a 20% Paulus response in 60% of patients at 3 weeks, but this was not sustained despite repeated infusions. In contrast, 60% of patients receiving higher doses (3 mg/kg and 10 mg/kg) maintained benefit for about 16 weeks and diminished thereafter.

In the presence of MTX the results were different. All 3 doses (1, 3, 10 mg/kg) gave a sustained response, which at the higher two doses was sustained from the last treatment at 14 weeks to the 26th week and end of the trial. The reasons for the difference are discussed later.

A phase III randomized, placebo-controlled study, ATTRACT (anti-TNF therapy of rheumatoid arthritis with concomitant therapy), included patients with active disease despite their use of relatively high dose MTX–median dose, 15 mg/week. Some 428 patients from Europe and United States and Canada were enrolled and randomized into 5 groups. They were all maintained on MTX (last dose carried forward) and were given either 3 or 10 mg/kg infliximab at 0, 2, and 6 weeks and then continued at either 4 or 8 weekly intervals. The control group received MTX plus placebo infusions. The five groups were well matched for disease duration, severity, and disease activity at baseline (84).

This trial is continuing for two years, and the results have been decoded at 6 months (for signs and symptoms) and at 1 year (for X-ray assessment of joint damage) and at 2 years. There was a rapid onset of response, with a significant improvement in symptoms and signs by 2 weeks, and the great majority of patients achieved the American College of Rheumatology 20 criteria of response by 6 weeks, but with further increments in the number of responders up to 1 year (Figure 2). Over 60–70% change in individual parameters of disease activity was

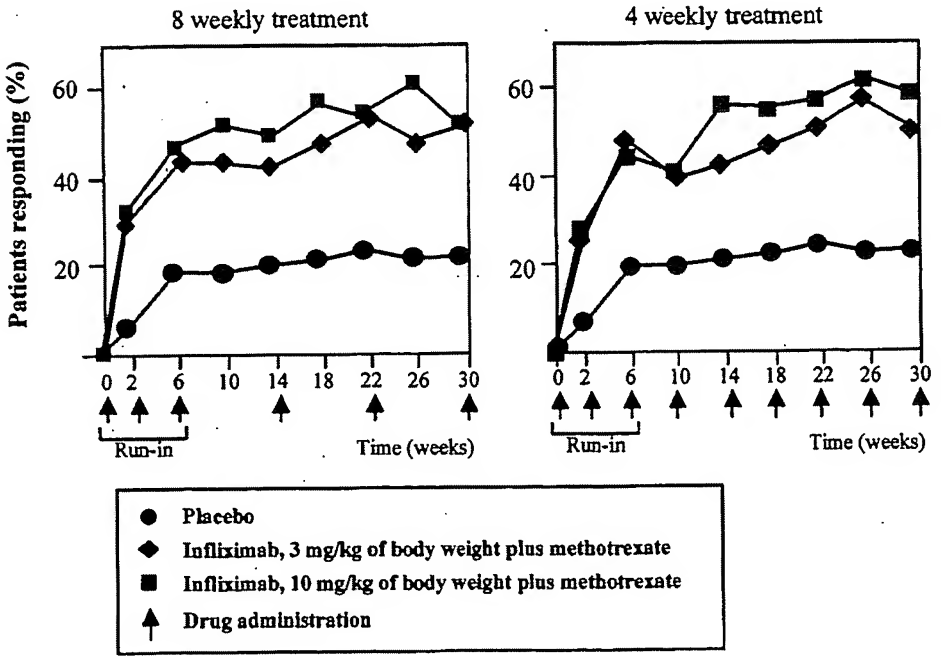

Figure 2 Efficacy of combination of infliximab and methotrexate versus methotrexate and placebo. Percentage of patients achieving a clinical response of 20% change from baseline as defined by the American College of Rheumatology criteria. Patients were treated with methotrexate (10–35 mg/week) and either placebo, 3 or 10 mg/kg inflixamab administered intravenously at time points indicated, in a DMARD unresponsive patient group with active disease despite methotrexate therapy (Maini et al; *Lancet* 1999–84).

achieved in the infliximab groups as compared to placebo. Between 50% and 60% of patients reached the 20% ACR criterion for improvement in all four active treatment groups. The sensitive biochemical marker of disease activity, CRP, reached normal limits within 2 weeks and remained at these concentrations throughout.

The 54-week end point of the ATTRACT trial was joint protection as judged by a change of deterioration in radiographs of the hands and feet, assessed by the Van der Heijde modification of the Sharp scoring system (85). This score attempts to assess cartilage and bone loss separately and has been shown to progress steadily during the disease, despite conventional (i.e. non anti-TNFα) therapy.

In the ATTRACT trial, the total Van der Heijde score (joint space narrowing and erosions in hands and feet) of the MTX alone group (placebo infusion) progressed as anticipated, with an increase in score of 4.0 (median) or 6.97 (mean). In contrast was a median score of 0.0 (mean 0.55) in the 340 patients treated with infliximab at various regimes. Zero change in the radiographic score from baseline in about 50% of the infliximab treated population was of interest as it occurred in patients who satisfied clinical responses assessed by the 20% ACR criteria as well as those who failed to do so. Moreover, the effect on halting of progression held true when the data were analyzed for a change in joint space narrowing and erosion counts separately (86) and subsequently at 2 years.

Results with CDP571, Humanized Anti-TNFα Monoclonal Antibody

The groups of Isenberg and of Panayi (87) reported that CDP571 was effective in RA patients at doses of 10 mg/kg, but not at 1 mg/kg. While this result was the first to confirm those obtained with infliximab, the CDP571 anti-TNFα appeared to be less effective than infliximab at equivalent doses. Its development seems to have been discontinued for RA, possibly because it appeared less effective; however, it is still in development for Crohn's disease (96).

D2E7 A Human Anti-TNFα Monoclonal Antibody

Whereas there are few publications with D2E7, a number of studies are published as abstracts. The overall picture is that D2E7 appears to be an effective anti-TNFα antibody, efficacious in RA by both subcutaneous and intravenous injection, over a range of doses. The clinical results so far appear to be comparable to those obtained with infliximab or etanercept (88–92). The mechanism of action studies so far indicate similar results to infliximab, for example, the reduction in IL-1 expression (93). Joint protection was described after long-term treatment (92).

TNFα Blockade with Etanercept (Enbrel™)

The efficacy of etanercept in RA has been demonstrated in a series of clinical trials that led to its approval by the FDA in November 1998. A dose ranging phase II study compared 0.25, 2, and 16 mg/m^2 etanercept subcutaneously twice a week for

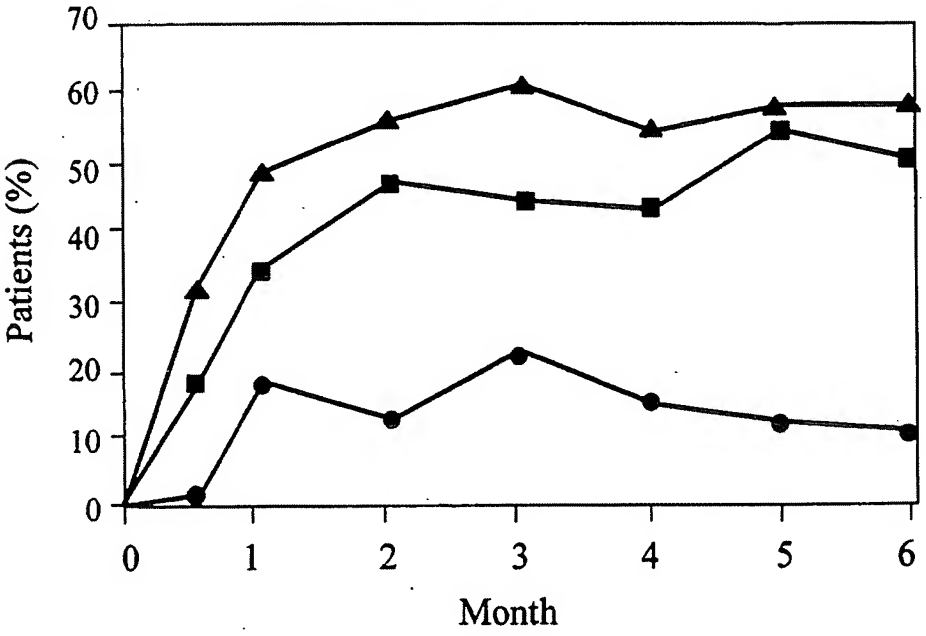

Figure 3 Efficacy of etanercept versus placebo. ACR 20% results in patients treated with two doses of etanercept or placebo injections administered subcutaneously twice weekly over a six-month period in DMARD unresponsive population. ▲ 25 mg etanercept, ■ 10 mg etanerce[t. • placebo (Moreland et al, *Ann. Internal Med.* 1999).

three months. The highest dose yielded approximately a 60% reduction in swollen and tender joints, compared to 25% with placebo (94).

In a further trial, etanercept was administered at 10 or 25 mg subcutaneously twice per week for 6 months and compared to placebo. The 25 mg dose was efficacious at 3 and 6 months; 59% showed an ACR 20 response at 6 months, compared to 11% with placebo (95) (Figure 3).

Etanercept was added to treatment with MTX in another randomized trial involving 89 patients with active disease despite MTX therapy: 71% of the patients receiving MTX plus etanercept responded, versus 27% in the MTX plus placebo (96). In this trial there was no treatment group which received etanercept alone, hence it is not clear whether MTX plus etanercept is more efficacious than etanercept alone, as was demonstrated in infliximab trials in which treatment groups receiving infliximab alone could be compared with infliximab co-administered with MTX (82).

Other studies have investigated progression in joint damage using etanercept. The results were presented at the November 1999 American College of Rheumatology Meeting. Both 10 mg and 25 mg etanercept subcutaneously twice weekly for 1 year were compared to MTX in patients with active RA of less than 3 years'

duration who had not previously been treated with methotrexate (97). Etanercept at 25 mg per week was at least as efficacious as MTX in controlling signs and symptoms and was better than MTX in retarding progression of erosions assessed by radiography of hands and feet in patients with erosions at base line, but not in patients without erosions on X-rays taken upon entry into the trial. No difference was noted in joint space narrowing. These data suggest that etanercept is more effective in controlling bone damage than cartilage damage in RA. Secondary end points were improved.

In a clinical trial on 69 children with juvenile severe poly-articular RA, treatment of patients with 0.4 mg/kg etanercept twice weekly for 90 days induced improvement in 74%. Over the next 9 months, half of the patients randomized to receive continuing etanercept had a disease relapse rate of 24%, in contrast to 77% of those receiving placebo (98). This result has led to the approval of etanercept for juvenile RA.

TNF-R p55 Fc (Lenercept)

This was the first TNF-R IgG Fc fusion protein to be extensively tested in the clinic in sepsis and then in RA. The results were positive, but were somewhat variable (99–102). The reasons for this inconsistency are not fully known, but two distinct hypotheses for this variation have been discussed. One is that there were batch-to-batch variations in the product, due to manufacturing problems. Another is that lenercept was more immunogenic in vivo than would have been predicted from its fully human derivation. Lesslauer and colleagues have reported that the extended peptide linker between the immunoglobulin Fc region and the TNF receptor was the immunogenic part of the molecule, and with time, antibodies generated extended to the TNF-R itself (103). If the latter hypothesis is correct, then agonistic effects due to crosslinking cell surface p55 receptors may have also contributed to variable results. It is noteworthy that Lesslauer reported agonistic antibodies in a small cohort (3 out of 7) of patients given subcutaneous lenercept, which would be more immunogenic. Changes in the half-life of lenercept were noted after the second injection compared to the first, which supports the concept that it was immunogenic. However in some clinical trials patients apparently showed sustained benefit over long periods of time.

PEG-ylated p55 TNF-R

This product has been shown to be active in rodent and primate models of arthritis and there is also preliminary evidence in rheumatoid arthritis phase I/II trials, which have been presented at meetings but not yet published in full (79, 104).

SAFETY OF ANTI-TNFα THERAPY

Overall, the available data both from clinical trials and less clearly from routine clinical practice suggest that the safety of TNFα inhibitors is at least as good as that of other anti-rheumatic drugs (34, 105, 106). In the clinical trial setting an increased

incidence of upper respiratory tract infections has been reported in comparison to the placebo control groups for both infliximab and etanercept. However, the higher rates of discontinuations in the placebo treatment arms of the trials led to shorter periods of observation. When adjusted to equivalent periods of exposure to anti-TNFα therapy in 'patient-years' these differences became insignificant. It should be noted that patients used in the trials were generally those with longstanding and severe RA, and such patients have increased rates of co-morbidity, complications, and shortened life-spans. However, despite the reassuring early data the risk of developing tuberculosis and fungal infections will require continuing surveillance.

The conclusion from clinical trials is that the overall safety profile is good. There were initial scares about a possibly increased incidence of lymphoma in the early infliximab studies. However, the incidence of lymphomas is higher than expected in rheumatoid patients with severe disease and receiving other immunosuppressant drugs. These features describe the type of patient included in the infliximab trials. The risk observed to date in all clinical trials in which patients were exposed to infliximab is not higher than might be expected (84), but longer-term follow-up of a larger number of patients is required to definitively exclude or confirm an association. As regards other cancers, the data of anti-TNFα studies have revealed that the number of nonlymphoid malignancies is similar to the expected frequency by comparison with populations of this age, using the NIH Cancer databases.

One complication of interest is that there appears to be a small risk of drug-induced lupus. The mechanism is poorly understood and may be similar to that implicated in the worsening of disease following administration of anti-TNFα antibody and IL-10 therapy (which inhibits TNFα synthesis) in the murine NZB/W model of systemic lupus erythematosus (SLE) (107). NZB/W mice show a genetic deficiency of TNFα (108), which is corrected by administration of low doses of TNFα. TNFα knockouts also develop elevated levels of anti-double stranded (ds) DNA antibody (109), emphasizing the role of TNFα in regulating autoimmunity. Increase in anti-ds DNA antibodies occurs in up to 15% of patients given anti-TNFα antibody or fusion proteins (87, 110, 111), but only about 0.2% of patients treated with infliximab develop symptoms of SLE (111). The few patients reported to date have responded well to discontinuation of the antibody treatment, with complete reversal of the lupus syndrome. These results indicate that SLE is a cytokine-dependent disease but at a different end of the spectrum from RA. The risk of induced SLE by TNFα blocking therapy is therefore small, not dissimilar to that observed in the past in RA patients treated with D-penicillamine and sulphasalazine, so it is not a limitation in clinical practice.

With considerable evidence from TNFα knockout mice and other models that TNFα is important in the innate immune response and in the induction of adoptive immune responses, it is not surprising that there is a concern that bacterial, viral, or parasitic infections may be increased in prevalence or severity in anti-TNFα treated patients. Thus far there are no statistically augmented risks of serious infection during the trials, although in some increased antibiotic usage in anti-TNFα groups suggests that there may be a small difference. What will happen on a longer time scale of treatment is not clear, but as etanercept has already been on the market

for over a year, the infectious risk does not appear to be a major issue. Whether different anti-TNFα agents have a different propensity to infection is not known. Safety is discussed in more detail in other reviews (32, 34).

MECHANISM OF ACTION OF ANTI-TNFα THERAPY

Overview

There are many reasons to study the mechanism of action of therapeutic agents, especially with biological agents, where the specificity of the drug is much easier to ascertain and verify than is the case for small organic chemicals. Study of the patients before and after therapy offers insights into the pathogenesis of the disease process.

This opportunity has been extensively seized with infliximab, and there are multiple papers published on mechanistic clinical studies using this antibody. It is not known whether the mechanism of action of a TNFα inhibitor like infliximab which is given at a high concentration and so yields a 'cytokine washout,' is the same as the effects of a TNFα inhibitor like etanercept, which is given repeatedly at lower doses leading to much lower blood levels. Thus infliximab blood levels reach to well over 100 μg/ml, whereas etanercept reaches steady state levels of 3 μg/ml.

Anti-TNFα Downregulates the Cytokine Cascade In Vivo

The effect of anti-TNFα on other 'downstream' cytokines in the RA synovial cultures such as IL-1 (23), GM-CSF and IL-6 (reviewed in 33) was a very important part of the rationale for anti-TNFα therapy, as it was based on studies of the diseased rheumatoid tissue, put into culture in the absence of extrinsic stimuli. It was thus of interest and importance to evaluate whether this inhibitory effect on other pro-inflammatory cytokines was also observed in vivo during clinical trials. The easiest cytokine to assay in vivo is IL-6, as there are elevated levels in RA patients, averaging about 100 pg/ml, and IL-6 is bioactive in serum.

Within a day of infliximab therapy, serum IL-6 concentrations fell to normal levels (112). This was not unexpected as it was already known that CRP, an inflammatory serum marker believed to be controlled chiefly by IL-6, normalized within a few days of treatment with anti-TNFα (73). The reduction of IL-6 is formal proof that TNFα regulates other pro-inflammatory cytokines. The speed and magnitude of the diminution of IL-6 within 24 h makes it likely to be a direct consequence of TNFα blockade on the cytokine network, rather than due to reduction in numbers of cells producing IL-6 in synovium, for example, which might be due to the indirect effects on leukocyte trafficking, or the killing of TNFα-producing cells that express surface TNF. These cells, chiefly macrophages, would also be the IL-6 producing cells.

Other evidence exists for downregulation of the other pro-inflammatory cytokines and chemokines by anti-TNF in vivo. A reduction in IL-8, MCP-1 and

VEGF has been described (113, 114), and the group of Kalden has reported down-regulation of serum IL-1 (115).

A key question is how the reduction in multiple pro-inflammatory cytokines influences manifestations of disease. TNFα has been reported to reduce neuropathic pain (116), and reduction in TNFα may reduce pain by mechanisms involving the central nervous system. Joint swelling is due to fluid as well as cellular infiltration, and reduction in VEGF concentrations, a cytokine that was also cloned as vascular permeability factor, is likely to involve reduction in VEGF-induced permeability and contribute to the rapid reduction of joint swelling within two weeks of anti-TNFα therapy. The reduction in TNFα and the consequent reduction in IL-1 would be expected to reduce the synthesis of MMP and other degradative enzymes production. Support for this mechanism was obtained by serial studies of serum pro MMP levels, before and after anti-TNFα. There was a marked reduction in proMMP-3 and of proMMP-1 after infliximab, probably reflecting reduced joint synthesis (117). It would be expected that joint destruction would diminish as was subsequently established in longer term clinical trials (see Clinical Efficacy of Anti-TNFα Infliximab and 86).

Infliximab Diminishes Leukocyte Trafficking into Joints

The reduction in TNFα and the consequent reduction in IL-1 would have marked effects on endothelial activation (23). The diminution in endothelial adhesion molecules after anti-TNFα therapy has been monitored in the infliximab trials. Two approaches were used, the most quantitative of which was to measure the serum concentrations of soluble adhesion molecules, ICAM-1, E-selectin, and VCAM-1. Among these, the levels of E-selectin, which is an endothelial specific molecule, most probably reflects the adhesive properties of blood vessels more closely than those of ICAM-1 or VCAM-1, which are also expressed on many other cells in the synovium. Significant reductions in serum E-selectin and ICAM-1 were indeed detected (118). Less quantitative, but more relevant to the joint disease, was a semi-quantitative immunohistological analysis of synovial biopsies before and after anti-TNFα therapy. This had demonstrated, to 'blinded' observers, that the expression of the adhesion molecules E-selectin, ICAM-1, and VCAM-1 in the synovium was diminished after anti-TNFα antibody therapy (119).

It was discussed above that serum levels of many but not all chemokines are reduced after infliximab therapy. Together with the adhesion molecule data, this strongly suggests that leucocyte trafficking will be reduced as leucocyte trafficking critically involves both adhesion molecules and chemokines. It was possible to verify this directly in a small clinical trial using ^{111}indium labelled autologous granulocytes, reinfused into the patient before and 2 weeks after infliximab treatment (114). This trial showed reductions in granulocyte influx of 40%–50%.

While the ^{111}indium granulocyte joint cell uptake results strictly apply only to the granulocytes, it is very likely that other leucocytes are also entering joints more slowly after infliximab therapy because the requirements for chemokines and

adhesion molecules for cell recruitment apply to all cells. Post-treatment synovial biopsies are much less cellular, with the numbers of T cells and macrophages reduced (119). Since T lymphocytes and macrophages also use the same spectrum of adhesion molecules as neutrophils, and relevant chemokines such as MCP-1 are downregulated, it is likely that there is reduced trafficking of all major leucocyte subsets into the joint. Cellularity is a balance between immigration and loss, and increased apoptosis, noted in the T lymphocytes areas (after infliximab therapy) may also contribute to the reduced cellularity found post treatment (P Taylor, unpublished data). It seems to do so in Crohn's disease after anti-TNFα therapy (Sander van Deventer, personal communication).

Infliximab Reduces VEGF and Angiogenesis in Inflamed Joints

Angiogenesis is a prominent feature of the chronic rheumatoid synovium, and so it was pertinent to investigate whether infliximab therapy was associated with reduced angiogenesis in order to investigate the relationship between inflammation and angiogenesis. Initial studies focused on measurement of VEGF, a potent and endothelial specific growth factor that promotes angiogenesis. Following the previous work of Fava and Koch, who had demonstrated high VEGF levels in rheumatoid synovium (120, 121), we assayed longitudinal blood samples from patients in the infliximab trials on the assumption that raised serum levels of VEGF might reflect enhanced synovial synthesis of VEGF. Serial synovial biopsies would not be possible in a sufficient percentage of patients to permit accurate statistical analysis. Pretreatment serum VEGF concentrations were indeed elevated; the degree of elevation correlated with a marker of disease activity, CRP, and was significantly reduced after infliximab therapy in two separate trials (113).

The partial reduction in serum VEGF levels led us to explore subsequently the possibility that angiogenesis was reduced in the synovium. Computerized image analysis of endothelium for multiple markers of endothelium (e.g. VWF, CD31) and neovasculature ($\alpha v\beta 3$) has shown a reduced vascularity after infliximab therapy (122).

Infliximab Restores the Hematological Abnormalities in RA

There are multiple hematological abnormalities in active RA. For example the counts of neutrophils tend to be on the high side. A tendency to low hemoglobin (Hb) concentration is common in RA patients. In view of the profound effects of multiple cytokines upregulated in RA on hemopoiesis, it was of interest to investigate the effects of infliximab on the blood constituents of RA patients in the infliximab clinical trials.

In the first phase II trial, with an end point at 4 weeks, Hb levels fell over this 4-week period in the placebo-treated group, possibly due to blood loss for experimental analyses. With the 1 mg/kg infliximab treatment it stayed at the same level, but at 10 mg/kg, the Hb level was significantly elevated over the

baseline. This result suggests that the anemia of RA, and by inference probably the anemia of other chronic inflammatory diseases, is cytokine dependent. Whether the anemia is an effect of TNFα or IL-6 or both is not clear, since both these cytokines are reported to diminish red cell production in certain in vitro systems (123).

Elevated platelet levels are a potentially dangerous consequence of RA, as elevated platelet levels may promote thrombotic and atherosclerotic complications. Infliximab diminished the elevated platelet levels to the normal range (81). The elevated fibrinogen levels commonly found in active RA patients are also likely to predispose to these diseases. However, there is as yet no evidence to support the hypothesis that infliximab is protective against coronary or cerebral thrombosis. The tendency of RA patients to have a moderate neutrophilia was normalized after infliximab therapy.

WHAT HAVE WE LEARNED?

Immunogenicity of Monoclonal Antibodies and Biologicals Is Variable and Can Be Contained

A real concern in the past has been the immunogenicity of antibodies. With murine monoclonals, this concern was borne out in clinical trials, for example of anti-IL-6, where initial efficacy rapidly waned (124). Subsequent antibodies were molecularly engineered to reduce the percentage of mouse sequences in an attempt to deal with this problem. Chimeric antibodies retain the mouse Fv antigen combining site, and thus they are three-quarters human (72). More complex techniques such as CDR grafting leading to humanized antibodies were designed to reduce potential immunogenicity further (125). With the phage display technique (126) it is possible to produce antibodies comprising only human sequences. This is also the case using transgenic mice that have had their Ig loci knocked out and replaced by human heavy and light chain loci (127, 128).

However, in all these cases it is possible to generate anti-idiotype antibodies directed against the binding site itself. This is well known, as the work of Oudin had demonstrated that autologous anti-idiotype antisera can be raised in rabbits (129). Hence the actual degree of immunogenicity of an antibody is difficult to predict. Fully human ones may still generate anti-idiotype or anti-allotype responses. Partly mouse ones, depending on which V regions are used, may not differ that much from the nearest human sequences.

The immunogenicity of antibodies may be reduced by a variety of procedures. First, there is evidence that aggregation of human antibodies augments their immunogenicity, just as was found previously in mice. Technical improvements in the preparation of the chimeric antibodies appear to have reduced the incidence of infusion reactions and diminished the incidence of antibody response to injected antibody (human anti-chimeric antibody—HACA) response.

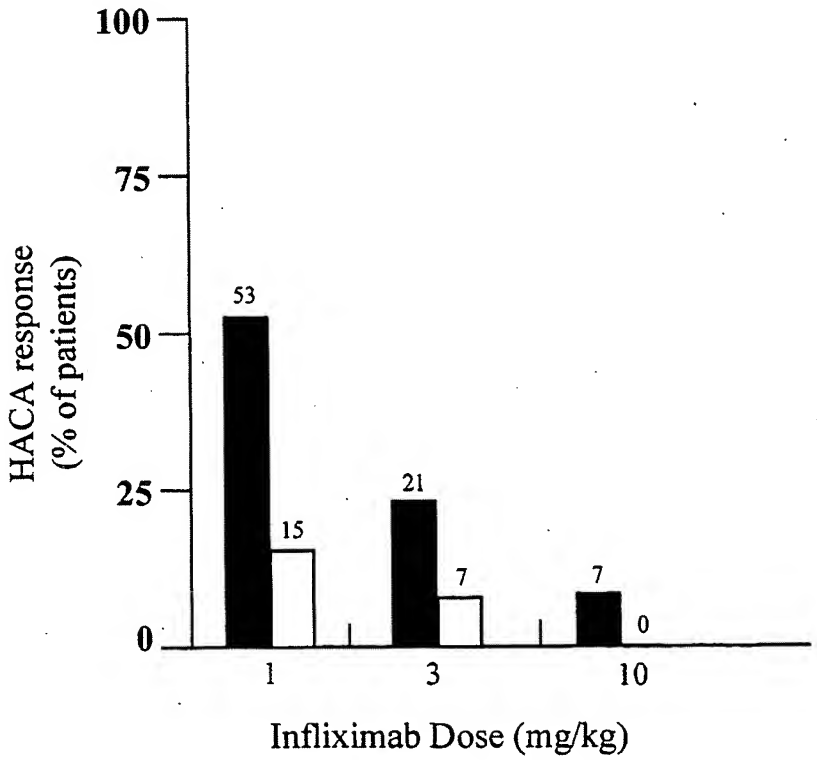

Figure 4 The incidence of human antichimeric antibodies (HACA) in patients treated with infliximab and methotrexate is inversely proportional to that of the dose of infleximab and is diminished in methotrexate. ■ infliximab alone; ❑ infliximab plus methotrexate (Maini et al, *Arthritis Rheum.* 1998).

Secondly, it is apparent that the frequency of the HACA response is inversely related to the amount of antibody infused (82). For example, 1 mg/kg of infliximab was much more frequently immunogenic than 3 mg/kg or 10 mg/kg (Figure 4). The mechanism for this inverse dose relationship appears to be high zone tolerance (HZT) extensively studied in the late 1960s, early 1970s. This was achieved by injection of high doses of soluble protein, and much of this old literature concerns deaggregated gamma globulin. HZT was described in many species (130–132), and whereas the inverse dose response is not formal proof of immunological tolerance, it is highly suggestive of HZT. The proof would require challenge with gamma globulin and adjuvant, which is clearly not possible.

Third, there is evidence that the frequency of the HACA response is diminished in the presence of methotrexate (MTX) (82, 133). This indicates that MTX has an immunosuppressive effect, as had been found by others (134).

With TNF-R fusion proteins, there have been variable results. Lenercept, the p55 TNF-R Fc fusion protein, was found to be highly immunogenic (103), and this contributed to its eventual abandonment. The first evidence for immunogenicity

noted was a reduction in the half-life after the first dose, from 7 to 4 days. Subsequently the antibody response was measured, and epitopes recognized were mapped by Lesslauer and his colleagues. They found that the earliest epitopes recognized were in the hinge region, which is extended and differs in conformation from that of native Ig. The epitopes recognized then extended to the p55 TNF-R (103). As most monoclonal antibodies to the p55 TNF-R are agonistic (135), it is possible that agonistic antibodies were raised and that they contributed to the variable results obtained.

An analogous situation has been documented in mice with gene therapy with an adenovirus encoding for a human p55 TNF-R mouse Fc fusion protein. This was initially efficacious, but subsequently the benefit was lost and the disease activity rebounded to a level worse than that of the controls. The sera of these mice had antibodies to human p55 TNF-R, some of which cross-reacted with murine TNF-R (136).

In contrast, initial reports suggested that etanercept was non-immunogenic, but with more sensitive assays, antibodies to etanercept were detected in 16% of treated individuals (137). However, it is reported that these do not interfere with chronic therapy.

Chronic Treatment with Antibodies and Fusion Proteins Is Possible

When antibody therapy was first conceived, it was not known whether antibodies were going to be used for short term or whether they could also be used long term (i.e. several years). This question has been resolved, and it is possible to use some antibodies or fusion proteins long term. Patients have been treated with such agents for up to one year in open-label continuation studies (100) and with infliximab in a randomized controlled trial (86).

Hence, long-term therapy is possible provided the therapy is efficacious and of low immunogenicity. The current regimes for infliximab—3 or 10 mg/kg infusion in the presence of MTX, or of etanercept—25 mg s.c. twice per week—appear to fulfill these criteria. D2E7, a human antibody produced by phage display, has also been used for over a year (92).

Why Are There No Cures with Anti-TNFα Therapy?

Remission is rare in late active RA (138). Nevertheless our concept that there is a disregulated cytokine equilibrium, (31, 33) suggests that if the balance was normalized, the augmented anti-inflammatory pathways may restore homeostasis. One reason for the lack of sustained benefit when therapy is stopped emerged from monitoring the effect of anti-TNFα therapy on the endogenous cytokine inhibitors. Serum IL-1Ra and soluble TNF-R were found to diminish after infliximab (112). There is a suggestion in a small group of patients that serum IL-10 was augmented (139). However, serum IL-10 levels are low and may not reflect the situation in the synovium, since IL-10 production in in vitro rheumatoid synovial cultures is inhibited by anti-TNFα antibody (58).

These results suggest that additional therapeutic benefit may ensue by restoring cytokine inhibitor levels in anti-TNFα–treated patients. Whether it might be achieved by co-administration of IL-10 or IL-1Ra is not clear. We have shown in murine collagen–induced arthritis that anti-TNFα and IL-10 work additively (140); Feige et al (141) have shown that in rat arthritis models the therapeutic effect of TNF-inhibitor (TNF-R PEG) is augmented by IL-1Ra.

Our overall conclusion is that the TNFα overproduction, while a key pathogenic mechanism in the disease (in human and mouse), is certainly not the only pathogenic pathway implicated in maintaining disease chronicity. Other pathways likely to be involved in maintenance of chronicity include augmented angiogenesis (37, 122, 142), lack of synovial apoptosis (12, 143–145), and abnormalities of the immune system (146). It will be important to target these mechanisms of chronicity if a cure is to be achieved.

Combination Therapy Works

Studies in experimental systems, undertaken in the past, mainly in the mouse collagen–induced arthritis model of RA, have demonstrated that the benefit of anti-TNFα therapy may be augmented by a variety of forms of anti–T cell therapy. The first to be documented in detail was synergy with anti-CD4 antibodies (83), but synergy can also be documented with blockade of costimulatory molecules (CD80 and CD86), using CTLA-4Ig fusion protein or anti-CD3 antibodies (Williams, unpublished data).

The extrapolation of these data into human disease, requiring the application of unlicensed drugs in clinical trials, is unrealistic. The first combination therapy targeting the immune system as well as TNFα was performed using MTX, and was highly successful (82). This has led to the routine use of infliximab with MTX (82, 84, 86). Increased efficacy without increased toxicity has also subsequently been found using etanercept (96).

The optimum combination therapy has not yet been evaluated, and it is possible that in human RA, as in the mouse, additional anti-T cell therapy, for example with anti-CD4 or anti-CD3 or cyclosporin, may lead to benefit in the patients not responding to anti-TNFα therapy alone.

Pathogenesis of Joint Damage

It has been proposed that contrary to the conventional view, inflammation and joint destruction may not be causally related. Evidence that they are uncoupled and due to different processes, with joint destruction due to an 'autonomous' tumorlike mass of fibroblasts, has been put forward (147). This was established by transplanting human fibroblast-like synoviocytes derived from rheumatoid tissue into immunocompromised SCID mice and demonstrating invasion and destruction of co-implanted cartilage. However, the clinical joint protection studies with infliximab, etanercept, and D2E7 indicate that this process of joint destruction is also likely dependent on TNFα (86, 92, 97).

The control of joint destruction, and hence the preservation of function, has been viewed as the holy grail in the therapy of rheumatoid arthritis. Randomized controlled trials have provided evidence that drugs such as MTX, leflunomide, and sulphasalazine retard structural damage compared with placebo therapy. In the ATTRACT phase III trial, the 12-month radiological analysis demonstrated that cartilage and bone damage (assessed by a modified Sharp score) progressed relentlessly in the group with active disease resistant to MTX who continue to receive MTX and placebo infusions, but was arrested in the groups receiving both MTX and infliximab in about 50% of patients (86). Etanercept used alone also had bone protective effects (97). Follow-up studies with D2E7 over one year have shown radiological benefit, although this was not a randomized and fully blinded prospective study (92).

The rush of recent clinical trial results clearly suggests that the current biological anti-TNFα agents have major protective effects against joint destruction. These data in RA are reminiscent of the protection of cartilage and bone with anti-TNFα antibody therapy in collagen II–induced arthritis in mice (66, 67) especially when combined with anti-T cell therapy (83). Prior to these animal studies, in vitro experiments had shown that TNFα was able to induce cartilage damage (148, 149) and bone damage (150).

The relative contribution of IL-1 and TNFα in mediating tissue destruction continues to be debated. The view has been espoused that targeted IL-1 blockade is critical in regulating joint destruction (151). This claim is based on a combination of in vitro and in vivo studies, which demonstrate the superior potency of IL-1 over TNFα in the induction of cartilage and bone damage (152, 153).

Experimental data exist, however, that can reconcile the role of TNFα and IL-1 in this regard. The addition of anti-TNFα antibody to RA synovial cells in vitro reduced IL-1 synthesis (23), and subsequently evidence was obtained that this occurs in vivo following therapy with infliximab (115, 154). Downregulation of IL-1 production in RA patients has also been recently reported in patients treated with D2E7 by Van den Berg's group (93). The work of Dinarello and others has highlighted the synergy between TNFα and IL-1 in many systems (29). Hence, it is possible that depletion of TNFα not only reduces production of IL-1 in joints, but also diminishes the synergistic effect of TNFα and IL-1.

Since anti-TNFα therapy combined with MTX has proved to show an unexpected degree of joint protective effect, it seems likely that the added benefit from co-administration of other anti-cytokines may be small. However, anti-IL-1 therapy may prove beneficial in patients unresponsive to anti-TNFα therapy (30%–40% in some trials) and in conjunction with anti-TNFα therapy administered at suboptimal levels (141).

The mechanisms of bone damage in RA are not fully understood. Osteoclasts are believed to play an important role in erosions of bone. The strongest signal for the differentiation and activation of osteoclasts is provided by RANK ligand (RANKL, also known as ODF, TRANCE, or OPG ligand), which is expressed by mesenchymal cells (e.g. synoviocytes and osteoblasts) as well as T lymphocytes.

TNFα may itself be able to induce osteoclast differentiation, by-passing RANK, in vitro (155). This result is not surprising as RANK (receptor activated inducer of NFκB) signals chiefly by inducing NFκB, as does TNFα. Whether anti-TNFα therapy has any impact on RANKL expression, or on the levels of the soluble inhibitor of osteoclast activation, osteoprotegerin (OPG), which blocks RANKL, is not known at present. This is certainly possible as TNFα and IL-1 have been described to upregulate both RANKL and OPG (156, 157). The effect of anti-TNFα therapy on the critical RANKL/OPG balance remains to be established.

Perhaps the most important conclusion from the three recent studies of anti-TNFα blockade on protecting joints (86, 92, 97) is the awareness that targeting TNFα is effective even in late-stage disease. Whether blocking RANKL by raising OPG levels will have additional benefits to blocking TNFα alone is not known, but in the rat adjuvant arthritis model, the administration of OPG was associated with joint protection (158).

CONCLUSIONS

Anti-TNFα therapy is one of the current successes of the biotechnology industry, which has cloned cytokines and generated inhibitors to them. It is also one of the successes of research in the cytokine field aimed at understanding the key, rate-limiting cytokines involved in the pathogenesis of disease, which might be suitable therapeutic targets. The success of anti-TNFα therapy in RA has prompted clinical studies in related diseases, such as Crohn's disease, which have also been highly successful therapeutically (159, 160), and more recently in the treatment of psoriatic arthritis and ankylosing spondylitis. We anticipate that this is the beginning of a wave of new therapies based on a rational understanding of the molecular pathogenesis of complex diseases. Many of these therapies will be targeted at cytokines. For maximum impact on improving the quality of life and secondary prevention of morbidity and premature mortality, cytokine-regulating drugs will need to be used in early stages of the disease. Durability of benefit, safety, and pharmacoeconomic issues will determine whether the early promise of the success of this knowledge-based approach will prove to be a boon to sufferers of incurable and painful diseases.

LITERATURE CITED

1. Stasny P. 1978. Association of the B cell alloantigen DRW4 with rheumatoid arthritis. *N. Engl. J. Med.* 298:869–71
2. Gregersen PK, Silver J, Winchester RJ. 1987. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum.* 30:1205–13
3. Todd JA, Acha-Orbea H, Bell JI, Chao N, Fronek Z, Jacob CO, McDermott M, Sinha

AA, Timmerman L, Steinman L, McDevitt HO. 1988. A molecular basis for MHC class II-associated autoimmunity. *Science* 240:1003–9
4. Laurence JS. 1970. Rheumatoid arthritis: nature or nurture? *Ann. Rheum. Dis.* 29: 357–69
5. Silman AJ, MacGregor AJ, Thomson W, Holligan S, Carthy D, Farhan A, Ollier WER. 1993. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br. J. Rheumatol.* 32:903–7
6. Erhardt CC, Mumford PA, Venables PJW, Maini RN. 1989. Factors predicting a poor life prognosis in rheumatoid arthritis: an eight year prospective study. *Ann. Rheum. Dis.* 48:7–13
7. Pincus T, Callahan LF. 1993. What is the natural history of rheumatoid arthritis? *Rheum. Dis. Clin. North Am.* 19:123–51
8. Maini RN, Zvaifler NJSE. 1994. Rheumatoid arthritis. In *Rheumatology*, ed. JH Klippel, PA Dieppe, 3.1. - 3.14.8. London: Mosby Year Book Europe
9. Smolen JS, Kalden JR, Maini RN, eds. 1992. *Rheumatoid Arthritis*. Berlin: Springer-Verlag
10. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA* 72:3666–70
11. Old LJ. 1985. Tumor necrosis factor (TNF). *Science* 230:630–32
12. Pennica D, Nedwin GE, Hayflick JS, P.H.S, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV. 1984. Human tumor necrosis factor: precursor structure expression and homology to lymphotoxin. *Nature* 312:724–29
13. Beutler B, Cerami A. 1988. Tumor necrosis, cachexia, shock, and inflammation: a common mediator. *Annu. Rev. Biochem.* 57:505–18
14. Vassalli P. 1992. The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* 10:411
15. Ruddle NH, Waksman BH. 1968. Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. I. Characterization of the phenomenon. *J. Exp. Med.* 128:1237–54
16. Gray PW, Aggarwal BB, Benton CV, Bringman TS, Henzel WJ, Jarrett JA, Leung DW, Moffat B, Ng P, Svedersky LP, Palladino MA, Nedwin GE. 1984. Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumor necrosis factor activity. *Nature* 312: 721–24
17. Vilcek J. 1998. The cytokines: an overview. In *The Cytokine Handbook*, ed. A. W. Thomson, pp. 1–20 Academic Press
18. Feldmann M, Dower S, Brennan FM. 1996. The role of cytokines in normal and pathological situations. In *Role of Cytokines in Autoimmunity*, ed. F. M. Brennan, M. Feldmann, pp. 1–23. Austin, TX: RG Landes Comp., Med. Intelligence Unit
19. Aggarwal BB, Samanta A, Feldmann M. 2000. TNFα. In *The Cytokine Reference*, ed. OJ, FM. New York: Academic Press.
20. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A. 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 330:662–4
21. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. 1997. A metalloproteinase disintegrin that releases tumour necrosis factor-alpha from cells. *Nature* 385:729–33
22. Fong Y, Tracey KJ, Moldawer LL, Hesse DG, Manogue KB, Kenney JS, Lee AT, Kuo GC, Allison AC, Lowry SF, Cerami A. 1989. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1β and interleukin 6 appearance during lethal bacteremia. *J. Exp. Med.* 170:1627–33

23. Brennan FM, Chantry D, Jackson A, Maini R, Feldmann M. 1989. Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* 2:244–47
24. Doukas J, Pober JS. 1990. IFN-γ enhances endothelial activation induced by tumor necrosis factor but not IL-1. *J. Immunol.* 145:1727–33
25. Schroder JM, Sticherling M, Henneicke HH, Preissner WC, Christophers E. 1990. IL-1α or tumor necrosis factor-α stimulate release of three NAP-1/IL-8-related neutrophil chemotactic proteins in human dermal fibroblasts. *J. Immunol.* 144:2223–32
26. Yokota S, Geppert T, Lipsky P. 1988. Enhancement of antigen- and mitogen-induced human T lymphocyte proliferation by tumor necrosis factor-α. *J. Immunol.* 140:531–36
27. Gordon C, Wofsy D. 1990. Effects of recombinant murine tumor necrosis factor-α on immune function. *J. Immunol.* 144: 1753–58
28. Cope AP, Londei M, Chu NR, Cohen SBA, Elliott MJ, Maini RN, Brennan FM, Feldmann M. 1994. Chronic exposure to tumor necrosis factor (TNF) in vitro impairs the activation of T cells through the T cell receptor/CD3 complex; reversal in vivo by anti-TNF antibodies in patients with rheumatoid arthritis. *J. Clin. Invest.* 94:749–60
29. Dinarello CA. 1994. The interleukin-1 family: 10 years of discovery. *FASEB J.* 8: 1314–25
30. Oppenheim JJ, Feldmann M, eds. 2000. *The Cytokine Reference – A Comprehensive Guide to the Role of Cytokines in Health and Disease.* London: Academic Press.
31. Feldmann M, Brennan FM, Maini RN. 1996. Rheumatoid arthritis. *Cell.* 85:307–10
32. Maini RN, Feldmann M. 2000. *Pocket Reference to TNFα Antagonism and Rheumatoid Arthritis.* London: Science Press.
33. Feldmann M, Brennan FM, Maini RN. 1996. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.* 14:397–440
34. Feldmann M, Elliott MJ, Woody JN, Maini RN. 1997. Anti TNFα therapy of rheumatoid arthritis. *Adv. Immunol.* 64: 283–350
35. Arend WP, Dayer J-M. 1995. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis. *Arthritis Rheum.* 38:151–60
36. Pisetsky DS. 2000. Tumor necrosis factor blockers in rheumatoid arthritis. *N. Engl. J. Med.* 342:810–11
37. Koch AE, Kunkel SL, Strieter RM. 1995. Cytokines in rheumatoid arthritis. *J. Invest. Med.* 43:28–38
38. Williams L, Gibbons DL, Gearing A, Feldmann M, Brennan FM. 1996. The synthetic metalloproteinase inhibitor BB-2275 blocks both p55 and p75 TNF receptor shedding, independent of its inhibitory effect on TNF processing. *J. Clin. Invest.* 97:2833–41
39. Seckinger P, Isaaz S, Dayer JM. 1988. A human inhibitor of tumor necrosis factor α. *J. Exp. Med.* 167:1511–16
40. Engelmann H, Aderka D, Rubinstein M, Rotman D, Wallach D. 1989. A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. *J. Biol. Chem.* 264:11974–80
41. Olsson I, Lantz S, Nilsson E, Peetre C, Thysell H, Grubb A, Adolf G. 1989. Isolation and characterization of a tumor necrosis factor binding protein from urine. *Eur. J. Haematol.* 42:270–75
42. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. 1993. Interleukin-10. *Annu. Rev. Immunol.* 11:165–90
43. Roberts AB, Sporn MB. 1990. The transforming growth factors-beta. In *Handbook of Experimental Pharmacology*, ed. M. B. Sporn, A. B. Roberts, 95:419–72. Heidelberg: Springer Verlag

44. Trepicchio WL, Bozza M, Pedneault G, Dorner AJ. 1996. Recombinant human interleukin-11 attenuates the inflammatory response through down-regulation of pro-inflammatory cytokine release and nitric oxide production. *J. Immunol.* 157:3627–34
45. Keith JC Jr, Albert L, Sonis ST, Pfeiffer CJ, Schaub RG. 1994. IL-11, a pleiotropic cytokine: exciting new effects of IL-11 on gastrointestinal mucosal biology. *Stem Cells.* 1:79–89
46. Dayer JM, Burger D. 1994. Interleukin-1, tumor necrosis factor and their specific inhibitors. *Eur. Cytokine Netw.* 5:563–71
47. Chu CQ, Field M, Feldmann M, Maini RN. 1991. Localization of tumor necrosis factor α in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheum.* 34:1125–32
48. Wood NC, Symons JA, Dickens E, Duff GW. 1992. In situ hybridization of IL-6 in rheumatoid arthritis. *Clin. Exp. Immunol.* 87:183–89
49. Saxne T, Palladino MA Jr, Heinegard D, Talal N, Wollheim FA. 1988. Detection of tumor necrosis factor α but not tumor necrosis factor β in rheumatoid arthritis synovial fluid and serum. *Arthritis Rheum.* 31:1041–45
50. Fontana A, Hentgartner H, Fehr K, Grob PJ, Cohen G. 1982. Interleukin-1 activity in the synovial fluid of patients with rheumatoid arthritis. *Rheumatol. Int.* 2: 49–56
51. Alvaro-Garcia JM, Zvaifler NJ, Brown CB, Kaushansky L, Firestein GS. 1991. Cytokines in chronic inflammatory arthritis. VI. Analysis of the synovial cells involved in granulocyte-macrophage colony stimulating factor production and gene expression in rheumatoid arthritis and its regulation by IL-1 and TNFα. *J. Immunol.* 146:3365–71
52. Hopkins SJ, Humphreys M, Jayson MI. 1988. Cytokines in synovial fluid. I. The presence of biologically active and immunoreactive IL-1. *Clin. Exp. Immunol.* 72:422–27
53. Di Giovine FS, Nuki G, Duff GW. 1988. Tumour necrosis factor in synovial exudates. *Ann. Rheum. Dis.* 47:768–72
54. Alvaro-Garcia JM, Zvaifler NJ, Firestein GS. 1989. Cytokines in chronic inflammatory arthritis. IV. GM-CSF mediated induction of class II MHC antigen on human monocytes: a possible role in RA. *J. Exp. Med.* 146:865–75
55. Malyak M, Swaney RE, Arend WP. 1993. Levels of synovial fluid interleukin-1 receptor antagonist in rheumatoid arthritis and other arthropathies. *Arthritis Rheum.* 36:781–89
56. Miossec P, Naviliat M, D'Angeac AD, Sany J, Banchereau J. 1990. Low levels of interleukin-4 and high levels of transforming growth factor β in rheumatoid synovitis. *Arthritis Rheum.* 33:1180–87
57. Leech M, Metz C, Hall P, Hutchinson P, Gianis K, Smith M, Weedon H, Holdsworth SR, Bucala R, Morand EF. 1999. Macrophage migration inhibitory factor in rheumatoid arthritis: evidence of proinflammatory function and regulation by glucocorticoids. *Arthritis Rheum.* 42:1601–8
58. Katsikis PD, Chu CQ, Brennan FM, Maini RN, Feldmann M. 1994. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. *J. Exp. Med.* 179:1517–27
59. Hermann JA, Hall MA, Maini RN, Feldmann M, Brennan FM. 1998. Important immunoregulatory role of interleukin-11 in the inflammatory process in rheumatoid arthritis. *Arthritis Rheum.* 41:1388–97
60. Allard SA, Muirden KD, Maini RN. 1991. Correlation of histopathological features of pannus with patterns of damage in different joints in rheumatoid arthritis. *Ann. Rheum. Dis.* 50:278–83
61. Zvaifler NJ, Firestein GS. 1994. Current comment: pannus and pannocytes: alternative models of joint destruction in rheumatoid arthritis. *Arthritis Rheum.* 37:783–89

62. Haworth C, Brennan FM, Chantry D, Turner M, Maini RN, Feldmann M. 1991. Expression of granulocyte-macrophage colony–stimulating factor in rheumatoid arthritis: regulation by tumor necrosis factor-alpha. *Eur. J. Immunol.* 21:2575–79
63. Deleuran BW, Chu CQ, Field M, Brennan FM, Mitchell T, Feldmann M, Maini RN. 1992. Localization of tumor necrosis factor receptors in the synovial tissue and cartilage-pannus junction in patients with rheumatoid arthritis. Implications for local actions of tumor necrosis factor alpha. *Arthritis Rheum.* 35:1170–78
64. Staines NA, Wooley PH. 1994. Collagen-induced arthritis—what can it teach us? *Br. J. Rheumatol.* 33:798–807
65. Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, Degott C, Kikutani H, Rajewsky K, Pasquali JL, Benoist C, Mathis D. 1999. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity* 10:451–61
66. Thorbecke GJ, Shah R, Leu CH, Kuruvilla AP, Hardison AM, Palladino MA. 1992. Involvement of endogenous tumour necrosis factor α and transforming growth factor β during induction of collagen type II arthritis in mice. *Proc. Natl. Acad. Sci. USA* 89:7375–79
67. Williams RO, Feldmann M, Maini RN. 1992. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* 89:9784–88
68. Piguet PF, Grau GE, Vesin C, Loetscher H, Gentz R, Lesslauer W. 1992. Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF receptor. *Immunology* 77:510–14
69. Wooley PH, Dutcher J, Widmer MB, Gillis S. 1993. Influence of a recombinant human soluble tumour necrosis factor receptor Fc fusion protein on type II collagen-induced arthritis in mice. *J. Immunol.* 151:6602–7
70. Breedveld FC. 2000. Therapeutic monoclonal antibodies. *Lancet* 355:735–40
71. Tugwell P. 2000. Pharmacoeconomics of drug therapy for rheumatoid arthritis. *Rheumatology* 39 (Suppl. 1):43–47
72. Knight DM, Trinh H, Le J, Siegel S, Shealy D, McDonough M, Scallon B, Moore AM, Vilcek J, Daddona P, Ghrayeb J. 1993. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Mol. Immunol.* 30:1443–53
73. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, Brennan FM, Walker J, Bijl H, Ghrayeb J, Woody JN. 1993. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum.* 36:1681–90
74. Sopwith M, Stephens S. 1999. An engineered human antibody for chronic therapy: CDP571. In *Biotechnology*, ed. H-J Rehn, G Reed, pp. 343–53. Weinham: Wiley-VCH. 2nd ed.
75. Jespers L, Roberts A, Mahler S, Winter G, Hoogenboom H. 1994. Guiding the selection of human antibodies from phage display repertoires to a single epitope of an antigen. *Biotechnology* 12:899–903
76. Hazleman B, Smith M, Moss K, Lisi L, Scott D, Sopwith M, Choy E, Isenberg D. 2000. Efficacy of a novel pegylated humanised anti-TNF fragment (CDP870) in patients with rheumatoid arthritis. *Rheumatology.* 39 (Suppl. 1):87
77. Mohler KM, Torrence DS, Smith CA. 1993. Soluble tumour necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J. Immunol.* 151:1548–61
78. Lesslauer W, Tabuchi H, Gentz R, Brockhaus M, Schlaeger EJ, Grau G, Piguet PF, Pointaire P, Vassalli P, Loetscher H. 1991. Recombinant soluble TNF receptor proteins protect mice from LPS-induced lethality. *Eur. J. Immunol.* 21:2883–86

79. Edwards CKI. 1999. PEGylated recombinant human soluble tumour necrosis factor receptor type I (r-Hu-sTNF-RI): novel high affinity TNF receptor designed for chronic inflammatory diseases. *Ann. Rheum. Dis.* 58 (Suppl 1):173–81
80. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Bijl H, Woody JN. 1994. Repeated therapy with monoclonal antibody to tumour necrosis factor α (cA2) in patients with rheumatoid arthritis. *Lancet* 344:1125–27
81. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, Woody JN. 1994. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet* 344:1105–10
82. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. 1998. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum.* 41:1552–63
83. Williams RO, Mason LJ, Feldmann M, Maini RN. 1994. Synergy between anti-CD4 and anti-TNF in the amelioration of established collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* 91:2762–66
84. Maini RN, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P, Group AS. 1999. Infliximab (chimeric anti-tumour necrosis factor a monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet.* 354:1932–39
85. van der Heijde DM. 1996. Plain X-rays in rheumatoid arthritis: overview of scoring methods, their reliability and applicability. *Baillieres Clin. Rheumatol.* 10:435–53
86. Lipsky P, St. Clair W, Furst D, Breedveld F, Smolen J, Kalden JR, Weisman M, Emery P, Harriman G, an der Heijde D, Maini RN. 1999. 54 week clinical and radiographic results from the attract trial: a phase III study of infliximab (RemicadeTM) in patient with active RA despite methotrexate (abstract). *Arthritis Rheum.* 42: S401
87. Rankin ECC, Choy EHS, Kassimos D, Kingsley GH, Sopwith SM, Isenberg DA, Panayi GS. 1995. The therapeutic effects of an engineered human anti-tumour necrosis factor alpha antibody (CD571) in rheumatoid arthritis. *Br. J. Rheumatol.* 34: 334–42
88. Kempeni J. 1999. Preliminary results of early clinical trials with the fully human anti-TNF monoclonal antibody D2E7. *Ann. Rheum. Dis.* 58 (Suppl. 1):I70–I2
89. van de Putte LBA, van Riel PLCM, den Broeder A, Sander O, Rau R, Binder C, Kruger K, Schattenkirchner M, Fenner H, Salfeld J, Bankmann Y, Kupper H, Kempeni J. 1998. A single dose placebo controlled phase I study of the fully human anti-TNF antibody D2E7 in patients with rheumatoid arthritis. *Arthritis Rheum.* 41: No..9 (Suppl.), Ab.:148:S57
90. Schattenkirchner M, Kruger K, Sander O, Rau R, Kroot E-J, van Riel PLCM, van de Putte LBA, den Broeder A, Fenner H, Kempeni J, Kupper H. 1999. Efficacy and tolerability of weekly subcutaneous injections of the fully human anti-TNF antibody D2E7 in patients with rheumatoid arthritis—results of a phase I study. *Arthritis Rheum.* 41: No. 9 (Suppl.), Ab.:149:S57
91. Rau R, Herborn G, Sander O, van de Putte LBA, van Riel PLC, den Broeder A, Schattenkirchner M, Wastlhuber J, Rihl M, Fenner H, Kempeni J, Kupper H. 1999. Long-term efficacy and tolerability of multiple i.v. doses of the fully human anti-TNF antibody D2E7 in patients with rheumatoid arthritis. *Arthritis Rheum.* 41(9):(Suppl):S55

92. Rau R, Herborn G, Sander O, van de Putte LBA, van Riel PLC, den Broeder A, Schattenkirchner M, Wastlhuber J, Rihl M, Fenner H, Kempeni J, Kupper H. 1999. Long term treatment with the fully human anti TNF antibody D2E7 slows radiographic disease progression in rheumatoid arthritis. *Arthritis Rheum.* 42:(9)(Suppl.):S400
93. Barrera P, Joosten LAB, den Broeder AA, van de Putte LBA, van den Berg WB. 1999. Effect of a fully human anti TNFα monoclonal antibody on the local and systemic expression of TNFα and IL-1β. *Arthritis Rheum.* 42:(9)(Suppl.) (Abst.) 29:S75
94. Moreland LW, Baumgartner SW, Schiff MH, Tindall eA, Fleischmann RM, Weaver AL, Ettlinger RE, Cohen S, Koopman WJ, Mohler K, Widmer MB, Blosch CM. 1997. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N. Engl. J. Med.* 337:141–47
95. Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, Weaver AL, Keystone EC, Furst DE, Mease PJ, Ruderman EM, Hortwitz DA, Arkfield DG, Garrison L, D.J B, Blosch CM, Lange ML, McDonnell ND, Weinblatt ME. 1999. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. *Ann. Intern. Med.* 130:478–86
96. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. 1999. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N. Engl. J. Med.* 340:253–59
97. Finck B, Martin R, Fleischmann R, Moreland L, Schiff M, Bathon J. 1999. A phase III trial of etanercept vs methotrexate (MTX) in early rheumatoid arthritis (Enbrel ERA trial). *Arthritis Rheum.* 42:S117
98. Lovell DJ, Giannini EH, Reiff PHA, Cawkwell GD, Silverman ED, Nocton JJ, Stein LD, Gedalia A, Ilowite NT, Wallace CA, Whitmore J, Finck BK. 2000. Etanercept in children with polyarticular juvenile rheumatoid arthritis. *N. Engl. J. Med.* 342:763–69
99. Sander O, Rau R, van Riel P, van de Putte L, Hasler F, Baudin M, Ludin E, McAuliffe T, Dickinson S, Kahny M-R, Lesslauer W, Van der Auwera P. 1996. Neutralization of TNF by Lenercept (TNFR55-IgG1, Ro 45-2081) in patients with rheumatoid arthritis treated for 3 months: results of a European phase II trial. *Arthritis Rheum.*, October 18–22, 1996, S242
100. Hasler F, van de Putte L, Baudin M, Lodin E, Durrwell L, McAuliffe T, van de Auwera P. 1996. Chronic TNF neutralization (up to 1 year) by lenercept (TNFR 55 IgG1, Ro 45-2081) in patients with rheumatoid arthitis: Results from open label extension of a double blind single-dose phase I study. *Arthritis Rheum.* 39(Suppl.):S243
101. Furst D, Weisman M, Paulus H, Bulpitt K, Weinblatt M, Polisson R, St. Clair P, Milnarik P, Baudin M, Ludin E, McAuliffe T, Kahry MR, Lesslauer W, Van de Auwera P. 1996. Neutralization of TNF by lenercept (TNFR 55 TgGt, Ro 45-2081) in patients with rheumatoid arthritis treated for 3 months: results of a phase II trial. *Arthritis Rheum.* S243
102. Cutolo M, Kirkham B, Bologna C, Sany J, Scott D, Books P, Forre O, Jain R, Kvien T, Markenson J, Seibold J, Sturrock R, Veys E, Edwards J, Zaug M, Durrwell L, Bisschops C, St Clair P, Milnarik P, Baudin M, Van der Auwera P. 1996. Loading/maintenance doses approach to neutralization of TNF by Lenercept (TNFR55-IgG1, Ro45-2081) inpatients with rheumatoid arthritis treated for 3 months: results of a double-blind placebo controlled phase II trial. *Arthritis Rheum.*, October 18–22, 1996, S243
103. Christen U, Thuerkauf R, Lesslauer W. 1998. Immunogenicity of a human

TNFR55-IgG1 fusion protein (Lenercept) in rheumatoid arthritis (RA) and multiple sclerosis (MS) patients. *J. Interfer. Cytokine Res.* 18:A121

104. Moreland LW, McCabe D, Caldwell JR, Sach M, Weisman M, Henry G, Seely J, Martin SW, Yee C, Bendele AM, Frazier J, Kohno T, Cosenza ME, Lyons S, Dayer J-M, Cohen A, Edwards CKI. 1999. Recombinant methionyl human tumor necrosis factor binding protein PEGylated dimer (TNFbp Dimer) in patients with active refractory rheumatoid arthritis. *J. Rheumatol.* In press
105. Moreland LW, Heck LW Jr, Koopman WJ. 1997. Biologic agents for treating rheumatoid arthritis. Concepts and progress. *Arthritis Rheum.* 40:397–409
106. Kavanaugh AF. 1998. Anti-tumor necrosis factor-α monoclonal antibody therapy for rheumatoid arthritis. *Emerging Therapies Rheumatoid Arthritis* 24:593–614
107. Ishida H, Muchamuel T, Sakaguchi S, Andrade S, Menon S, Howard M. 1994. Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* 179:305–10
108. Jacob CO, McDevitt HO. 1988. Tumour necrosis factor-alpha in murine autoimmune 'lupus' nephritis. *Nature* 331:356–58
109. Ettinger R, Daniel N. 2000. TNF-deficient mice develop anti-nuclear autoantibodies (abs). *Scand. J. Immunol.* 51 (Suppl.):88
110. Maini RN, Elliott MJ, Charles PJ, Feldmann M. 1994. Immunological intervention reveals reciprocal roles for TNFα and IL-10 in rheumatoid arthritis and SLE. *Springer Semin. Immunopathol.* 16:327–36
111. Charles PJ, Smeenk RJT, DeJong J, Feldmann M, Maini RN. 2000. Assessment of antibodies to dsDNA induced in rheumatoid arthritis (RA) patients following treatment with infliximab, a monoclonal antibody to TNF alpha. *Arthritis Rheum.* In press
112. Charles P, Elliott MJ, Davis D, Potter A, Kalden JR, Antoni C, Breedveld FC, Smolen JS, Eberl G, deWoody K, Feldmann M, Maini RN. 1999. Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. *J. Immunol.* 163:1521–28
113. Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M, Maini RN. 1998. Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis. *Arthritis Rheum.* 41:1258–65
114. Taylor PC, Peters AM, Paleolog E, Chapman PT, Elliott MJ, McCloskey R, Feldmann M, Maini RN. 2000. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor α blockade in patients with rheumatoid arthritis. *Arthritis Rheum.* 43:38–47
115. Lorenz HM, Antoni C, Valerius T, Repp R, Grunke M, Schwerdtner N, Nusslein H, Woody J, Kalden JR, Manger B. 1996. In vivo blockade of TNF-alpha by intravenous infusion of a chimeric monoclonal TNF-alpha antibody in patients with rheumatoid arthritis - Short term cellular and molecular effects. *J. Immunol.* 156:1646–53
116. Ignatowski TA, Covey WC, Knight PR, Severin CM, Nickola TJ, Spengler RN. 1999. Brain-derived TNF alpha mediates nueropathic pain. *Brain Res.* 841:70–77
117. Brennan FM, Browne KA, Green PA, Jaspar JM, Maini RN, Feldmann M. 1997. Reduction of serum matrix metalloproteinase 1 and matrix metalloproteinase 3 in rheumatoid arthritis patients following anti- tumour necrosis factor-alpha (cA2) therapy. *Br. J. Rheumatol.* 36:643–50
118. Paleolog EM, Hunt M, Elliott MJ, Feldmann M, Maini RN, Woody JN. 1996. Deactivation of vascular endothelium by

monoclonal anti-tumor necrosis factor α antibody in rheumatoid arthritis. *Arthritis Rheum.* 39:1082–91

119. Tak PP, Taylor PC, Breedveld FC, Smeets TJM, Daha MR, Kluin PM, Meinders AE, Maini RN. 1996. Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor α monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum.* 39:1077–81
120. Fava RA, Olsen NJ, Spencer-Green G, Yeo KT, Yeo TK, Berse B, Jackman RW, Senger DR, Dvorak HF, Brown LF. 1994. Vascular permeability factor/endothelial growth factor (VPF/VEGF): accumulation and expression in human synovial fluids and rheumatoid synovial tissue. *J. Exp. Med.* 180:341–46
121. Koch AE, Harlow LA, Haines GK, Amento EP, Unemori EN, Wong WL, Pope RM, Ferrara N. 1994. Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. *J. Immunol.* 152:4149–56
122. Maini RN, Taylor PC, Paleolog E, Charles P, Ballara S, Brennan FM, Feldmann M. 1999. Anti-tumour necrosis factor specific antibody (infliximab) treatment provides insights into the pathophysiology of rheumatoid arthritis. *Ann. Rheum. Dis.* 58(Suppl.) 1:156–60
123. Davis D, Charles PJ, Potter A, Feldmann M, Maini RN, Elliott MJ. 1997. Anaemia of chronic disease in rheumatoid arthritis: in vivo effects of tumour necrosis factor a blockade. *Br. J. Rheumatol.* 36:950–56
124. Wijdenes J, Racadot E, Wendling D. 1994. Interleukin 6 antibodies in rheumatoid arthritis. *J. Interferon Res.* 14:297–98
125. Soderlind E, Ohlin M, Carlsson R. 1999. Complementarily-determining region (CDR) implantation: a theme of recombination. *Immunotechnology* 4: 279–85
126. Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR. 1994. Making antibodies by phage display technology. *Ann. Rev. Immunol.* 12:433–55
127. Fishwild DM, O'Donnell SL, Bengoechea T, Hudson DV, Harding F, Bernhard SL, Jones D, Kay RM, Higgins KM, Schramm SR, Lonberg N. 1996. High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice. *Nat. Biotechnol.* 14:845–51
128. Green LL, Hardy MC, Maynard-Currie CE, Tsuda H, Louie DM, Mendez MJ, Abderrahim H, Noguchi M, Smith DH, Zeng Y, et al. 1994. Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. *Nat. Genet.* 7:13–21
129. Oudin J, J. M. 1963. A new form of allotype of rabbit gamma-globulins apparently correlated with antibody function and specificity C. *R. Acad. Sci.* 257:805–8
130. Dresser DW, Mitchison NA. 1968. The mechanism of immunological paralysis. *Adv. Immunol.* 8:129–81
131. Basten A, Miller JF, Sprent J, Cheers C. 1974. Cell-to-cell interaction in the immune response. X. T-cell-dependent suppression in tolerant mice. *J. Exp. Med.* 140:199–217
132. Chiller JM, Habicht GS, Weigle WO. 1970. Cellular sites of immunologic unresponsiveness. *Proc. Natl. Acad. Sci. USA* 65:551–56
133. Kavanaugh AF, Cush JJ, St Clair EW, McCune WJ, Braakman TAJ, Nichols LA, Lipsky PE. 1996. Anti-TNFα monoclonal antibody (mAb) treatment of rheumatoid arthritis (RA) patients with active disease on methotrexate (MTX): results of a double-blind, placebo controlled multicenter trial. *Arthritis Rheum.* 39:A575
134. Genestier L, Paillot R, Fournel S, Ferraro C, Miossec P, Revillard JP. 1998. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. *J. Clin. Invest.* 102:322–28

135. Espevik T, Brockhaus M, Loetscher H, Nonstad U, Shalaby R. 1990. Characterization of binding and biological effects of monoclonal antibodies against a human tumor necrosis factor receptor. *J. Exp. Med.* 171:415–26
136. Quattrocchi E, Dallman M, Feldmann M. 2000. Adenovirus mediated gene transfer of CTLA-4Ig fusion protein suppre sses experimental autoimmune arthritis. *Arthritis Rheum.* 43:1688–97
137. Garrision L, McDonnell ND. 1999. Etanercept: theapeutic use in patients with rheumatoid arthritis. *Ann. Rheum. Dis* 58 (suppl 1):165–69
138. Pinals RS, Masi AT, Larsen RA. 1981. Preliminary criteria for clinical remission in rheumatoid arthritis. *Arthritis Rheum.* 24:1308–15
139. Ohshima S, Saeki Y, Mima T, Suemura M, Ishida H, Shimizu M, McClosky R, Kishimoto T. 1996. Possible mechanism for the long-term efficacy of anti-TNF alpha antibody (cA2) therapy in RA. *Arthritis Rheum.* 39, No. 9 (Suppl.):S242
140. Walmsley M, Katsikis PD, Abney E, Parry S, Williams RO, Maini RN, Feldmann M. 1996. Interleukin-10 inhibition of the progression of established collagen-induced arthritis. *Arthritis Rheum.* 39:495–503
141. Feige U, Hu YL, Julian E, Duryea D, Bolon B. 1999. Combining anti IL-1 and anti-TNF treatments provides better efficacy in rat adjuvant arthritis than does either agent alone. *Arthritis Rheum.* 42:S383:(A1875)
142. Paleolog E. 1996. Angiogenesis: a critical process in the pathogenesis of RA - a role for VEGF? *Br. J. Rheumatol.* 35:917–20
143. Hayashida K, Shimaoka Y, Ochi T, Lipsky PE. 2000. Rheumatoid arthritis synovial stromal cells inhibit apoptosis and upregulate Bcl-xL expression by B cells in a CD49/CD29-CD106-dependent mechanism. *J. Immunol.* 164:1110–16
144. Salmon M, Scheel-Toellner D, Huissoon AP, Pilling D, Shamsadeen N, Hyde H, D'Angeac AD, Bacon PA, Emery P, Akbar AN. 1997. Inhibition of T cell apoptosis in the rheumatoid synovium. *J. Clin. Invest.* 99 (3):439–46
145. Aupperle KR, Boyle DL, Hendrix M, Seftor EA, Zvaifler NJ, Barbosa M, Firestein GS. 1998. Regulation of synoviocyte proliferation, apoptosis, and invasion by the p53 tumor suppressor gene. *Am. J. Pathol.* 152:1091–98
146. Brennan FM, Hayes AL, Ciesielski CJ, Foxwell BMJ, Feldmann M. 2000. Cytokine activated T cells are important in TNFα synthesis in rheumatoid arthritis: P13 kinase and NF-κB pathways discriminate between cytokine and TCR activated T cells. Submitted
147. Muller-Ladner U, Gay RE, Gay S. 1998. Molecular biology of cartilage and bone destruction. *Curr. Opin. Rheumatol.* 10:212–19
148. Saklatvala J, Sarsfield SJ, Townsend Y. 1985. Pig interleukin -1. Purification of two immunologically different leukocyte proteins that cause cartilage resorption, lymphocyte activation and fever. *J. Exp. Med.* 162:1208–15
149. Saklatvala J. 1986. Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature* 322:547–49
150. Gowen M, Mundy GG. 1986. Actions of recombinant interleukin 1, interleukin 2, and interferon gamma on bone resorption in vitro. *J. Immunol.* 136:2478–82
151. van den Berg WB. 1998. Joint inflammation and cartilage destruction may occur uncoupled. *Springer Semin. Immunopathol.* 20:149–64
152. Henderson B, Pettipher ER. 1989. Arthritogenic actions of recombinant IL-1 and tumour necrosis factor α in the rabbit: evidence for synergistic interactions between cytokines *in vivo*. *Clin. Exp. Immunol.* 75:306–10

153. Joosten LA, Helsen MM, Saxne T, van De Loo FA, Heinegard D, van Den Berg WB. 1999. IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation. *J. Immunol.* 163:5049–55
154. Ulfgren A-K, Andersson U, Engstrom M, Klareskog L, Maini RN, Taylor PC. 2000. Systemic anti-TNFα therapy in rheumatoid arthritis down-regulates synovial TNFα synthesis. Submitted
155. Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Morinaga T, Higashio K, Martin TJ, Suda T. 2000. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *J. Exp. Med.* 191:275–86
156. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell.* 93:165–76
157. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu a, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. 1998. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc. Natl. Acad. Sci. USA* 95:3597–3602
158. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM. 1999. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* 402:304–9
159. Present DHR, Targan P, Hanauer S, Mayer SB, van Hogezand L, Podolsky RA, Sands DK, Braakman TAJ, DeWoody KC, Schaible TF, van Deventer SJH. 1999. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N. Engl. J. Med.* 340:1398–1405
160. van Dulleman HM, van Deventer SJ, W. HD, Bijl HA, Jansen J, Tytgat GN, Woody J. 1995. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* 109:129–35